PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	29.03.2001
Applicant's or agent's file reference 19290P WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/09440	03/12/1999	03/12/1998

Applicant
MAX-PLANCK-GESELLSCHAFT...et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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PATENT COOPERATION TREAT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
19290P WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/09440	03/12/1999	03/12/1998
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant MAX-PLANCK-GESELLSCHAFT...et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/06/2000	29.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Vix, O Telephone No. +49 89 2399 7326

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/09440

I. Basis of the r port

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-51 as originally filed

Claims, No.:

1-40 with telefax of 14/12/2000

Drawings, sheets:

1/14-14/14 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 24-28, 29-36.

because:

☒ the said international application, or the said claims Nos. 24-28 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 29-36.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 10-14,17-18 22-23

	No:	Claims	1-9,15-16,19-21,37-40
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-23,37-40
Industrial applicability (IA)	Yes:	Claims	1-23,37-40
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: EP-A-0 614 978 (ROUSSEL-UCLAF) 14 September 1994 (1994-09-14)

D2: US-A-5 623 053 (LOUIS N. GASTINEL ET AL.) 22 April 1997 (1997-04-22)

D3: WO 95 09002 A (UNIVERSITY OF PENNSYLVANIA) 6 April 1995 (1995-04-06)

D4: EP-A-0 791 653 (SCHERING BIOTECH CORPORATION) 27 August 1997 (1997-08-27)

D5: JÉRÔME GALON ET AL.: 'Ligands and biological activities of soluble Fcgamma receptors' IMMUNOLOGY LETTERS, vol. 44, January 1995 (1995-01), pages 175-181,

D6: EP-A-0 321 842 (KISHIMOTO, TADAMITSU) 28 June 1989 (1989-06-28)

D7: JANET M. ALLEN ET AL.: 'Isolation and expression of functional high-affinity Fc receptor complementary DNAs' JOURNAL OF CRYSTAL GROWTH, vol. 93, no. 1-4, November 1988 (1988-11) - December 1988 (1988-12), pages 378-381, Amsterdam, NL

D8: WILHELM P. BURMEISTER ET AL.: 'Crystal structure at 2.2A resolution of the MHC-related neonatal Fc receptor' NATURE, vol. 372, no. 6504, 24 November 1994 (1994-11-24), pages 336-343, LONDON GB

D9: WO 99 05271 A (GOULD, HANNAH ET AL.) 4 February 1999 (1999-02-04)

D10 FR-A-2 739 560 (ROUSSEL UCLAF) 11 April 1997 (1997-04-11)

Document cited by the examiner:

D11 Grueninger-Leitch F, D'Arcy A, D'Arcy B, Chene C., 'Deglycosylation of proteins for crystallization using recombinant fusion protein glycosidases.', Protein Sci 1996 Dec;5(12):2617-22

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Concerning claims 24-28 applicant's attention is drawn to Rule 67(v) PCT. Claims 24-28 refer to a crystalline preparation for the generation of FcR crystal structure data. The subject-matter of claims 24-28 dealing with the presentation of crystal structure data and use thereof is not regarded as patentable invention within the meaning of

Rule 67 (v) PCT since it only relates to a presentation of information (protein structure coordinates) stored on a computer, including electronic databases and paper. The applicant is, however, requested to note that it may be possible to obtain alternative protection in the form of a copyright.

2. No examination has been performed for the subject-matter of claims 29-36 because of non-establishment of search report for said claims.
Relating to this, a meaningful opinion with regard to novelty of claims 29-36 cannot be established because it is not possible to determine if any of the presently known substances is falling under the terms of these claims. Besides it is noted, that the compounds of claims 29-34, and use their in claims 35-36, are not rendered novel just because of the fact that they have been identified by using a three-dimensional structure, e.g. such compounds can already exist. (Apart from this, it is also not possible to establish the scope of these claims without testing all known substances, clearly an undue burden).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Priority documents have not been available at the time of establishing this preliminary opinion. The IPER has been established under the assumption of valid priority rights. Should this however not be the case, the document D9 cited in the ISR as P-document might become important.

2. Novelty (Art. 33(2) PCT)

The application relates to recombinant soluble Fc receptors (FcR) and use thereof, the FcR being characterised by the absence of transmembrane domain, signal peptide and glycosylation.
The ISR reports an abundant prior art dealing with various type of soluble human or murine FcR and their use.
Due to the broad wording of the product claims 1-5, many known FcRs mentioned might fall within the scope of said claims. Although different specific examples or

embodiments in D1-D2 and D4 are related to eukaryotic expression system, all these documents also refer to prokaryotic expression system as other possible systems (e.g. D1 page 5, or D2 column 7 line 25-32, D4 column 7 line 37-40 and column 8, line 54-59). For instance, D1 discloses a FcγRIII human soluble receptor that can be expressed in both prokaryotic or eukaryotic system (D1 page 5). As such, this soluble FcγRIII attacks novelty of present claims 1-5 because it lacks the transmembrane domain, is not glycosylated when expressed in the prokaryotic system, and does not have a signal peptide.
Consequently, claims 1-5 can be considered as lacking novelty in view of the alternatively expressed Fc receptors disclosed in D1-D2 and D4.

Furthermore, it is to be mentioned that the specific sequences SEQ ID N°:1-6 mentioned in claim 5 show a high level of identity with different sequences deposited in public databases:
- SEQ ID N°1 shows 100% identity in a 168 amino-acids overlap with FcγRI (entry number P12314)
- SEQ ID N°2 shows 100% identity in a 170 amino-acids overlap with FcγRIIa (entry number P07446)
- SEQ ID N°3 shows 100% identity in a 184 amino-acids overlap with FcγRII (entry number 000523)
- SEQ ID N°4 shows 100% identity in a 175 amino-acids overlap with FcγRII (entry number R61122)
- SEQ ID N°5 shows 99.5% identity in a 183 amino-acids overlap with FcεRI (entry number R42336)
- SEQ ID N°6 shows 100% identity in a 274 amino-acids overlap with soluble CD23 (entry number W13143).

Correspondingly, the polynucleotides coding for the above mentioned FcRs are closely related to the subject-matter of claims 6-9. Thus, novelty of claims 6-9, which are directed to recombinant nucleic acid encoding FcR contained in a prokaryotic expression system, is questionable and cannot be acknowledged.

Claims 10-14 refer to a process for the determination of the amount of antibodies of a "certain Ig class in the blood" using the sFcR in an immuno assay. No corresponding examples appear to be cited in the available prior art.

However, claims 15-16 refers to a process for the determination of the "immune status" (see also item VIII) of patients with chronic diseases of the immune system (e.g. such as AIDS or MM) using the FcR in a competitive immunoassay. The prior art describes competition immuno-assays using soluble FcR (e.g. D3 page 20, line 9-11). Moreover, D1 (page 7 line 10-15) mentions the use of ELISA techniques in order to determine the amount of soluble FcR in biological fluids in order to diagnose "FcR- related" diseases. Thus, due to the broadly defined subject-matter, novelty of claims 15-16 cannot be acknowledged.

The pharmaceutical compositions of claims 19-21 containing soluble FcR also lack novelty because the polypeptides are known to be involved in various diseases such as autoimmune disorders and are used as active ingredients in pharmaceutical compositions - insofar the prokaryotic expression system is considered (e.g see D3 or D5 for autoimmune diseases, or D10 for HIV infection prevention).

Claims 22-23 refer to a crystalline preparation of a soluble recombinant Fc receptor and the generation of crystal structure data. No other sFcR of the available prior art is dealing with this specific subject-matter. Only D8 reports the structure of another class of Fc receptor which does not belong to the immunologically active Fc receptor of the present application. D8 deals with the soluble extracellular portion of rat neonatal FcR (FcRn) which has been successfully expressed and purified - the FcRn preparation allowed the crystallization of the protein in space group $C222_1$ prior to its structure determination at 2.2 A resolution. Thus, the subject matter of claims 22-23 is considered to be novel in view of the available prior art.

Claims 37-38 refer to a FcR bound to a solid phase whereas claims 39-40 use said solid phase support to adsorb immunoglobulin from different mixtures. Such claims are not novel in view of D2 which mentions such FcR attachment to a surface and the use of the attached immobilized FcR to isolate antibodies from a mixture comprising such antibodies (see D2 column 6, line 65 - col. 7, line 2). Thus said claims 37-40 are not considered to be novel in view of D2.

In summary, the subject matter of claims 1-9, 15-16, 19-21 and 37-40 is not considered to be novel. Thus, said claims do not meet the requirement of Articles 33(2)/(3) PCT.

3. Inventive step (Art. 33(3) PCT)

If at all new, the subject-matter of the claims 1-23 and 37-40 does not involve an inventive step for the following reasons.

3.1 The protein crystallization is a known bottle neck in 3D structure determination and needs a great amount of homogeneous biological material. Thus, the most common strategy in protein crystallography was to choose the "easiest" expression system over the more complex one. Therefore, the person of skill would choose to overexpress a protein to crystallize in a prokaryotic system, where the problems of glycosylation heterogeneity are limited (in addition, the prokaryotic system allows a high level of expression at limited cost). However, it is very often when the prokaryotic system expression does not allow a proper folding of the protein, or if the protein is not biologically active in its unglycosylated state, that the person skilled in the art would in priority select an eukaryotic expression system. In this case, the skilled person would carefully analyse the level of glycosylation of the protein expressed because it can be an important source of heterogeneity- or even hinder the interaction with different biological counterparts (if heavy glycosylation). Relating to this, in D11 the authors describe the importance of deglycosylation for the obtention of high quality protein crystals when the protein is produced in eukaryotic system.

In the present application, no examples of possible surprising effects or advantages of the prokaryotic system over the eukaryotic system has been demonstrated.

Therefore, in absence of evidence showing unknown or unexpected effects or properties of presently claimed sFcR, the presence of an inventive step cannot be acknowledged since the mere provision of sFcR produced in a prokaryotic system, using methods which are known in the art for this purpose, does not involve an inventive activity to a person skilled in the art (Art. 33(3) PCT).

Thus the subject-matter of claims 1-23 and 37-40 does not appear to be inventive over the available prior art D1-D6 describing the production and use of sFcR produced in eukaryotic expression systems. Consequently said claims do not satisfy the criterion set forth in Article 33(3) PCT.

3.2. Claims 10-14 relate to a process for determination of the amount of IgE or IgG

antibodies in the blood, plasma or serum of patient using ELISA. The prior art disclose various ELISA tests performed with immobilised antibodies to check the presence of Fc receptors in a media (e.g. D6, column 15). Due to the known biological function of the Fc receptor, and in view of the above mentioned existing tests, the subject-matter of claims 10-14 using the FcR to determine the presence of specific antibody (could be considered as a "mirror" ELISA test) does not appear to involve an inventive step in the sense of Art. 33(3) PCT.

3.3. Methods of modulating the interaction of immune complexes with Fc receptors were disclosed in D3 in order to treat diseases resulting from interaction between immune complexes and Fc receptors. Moreover, it was known that the FcRs are involved in various chronic diseases of the immune system (see also D4, column 1, line 41-51). Thus, the man skilled in the art would regard as a normal design procedure to screen substances in view of their ability to act as inhibitors of the FcR recognition in the cases where the FcR is implicated in chronical diseases such as autoimmune diseases. Thus, the subject-matter of claim 17-18 does not involve an inventive step and does not satisfy the criterion set forth in Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. The designations "soluble Fc receptor" or "FcγR" and "FcεR" appearing in claims 1-4 are not suitable to clearly and unambiguously characterise a specific polypeptide. Therefore, it needs to be clarified to clearly define the subject-matter of said claims. Relating to this, applicant's attention is drawn to the fact that a polypeptide is a chemical compound which can be clearly and unambiguously characterised by its primary sequence.

2. The expression "immune status" in claim 15 is ambiguous an open to interpretation, and thus render the scope of said claims unclear (Article 6 PCT).

Fig. 1

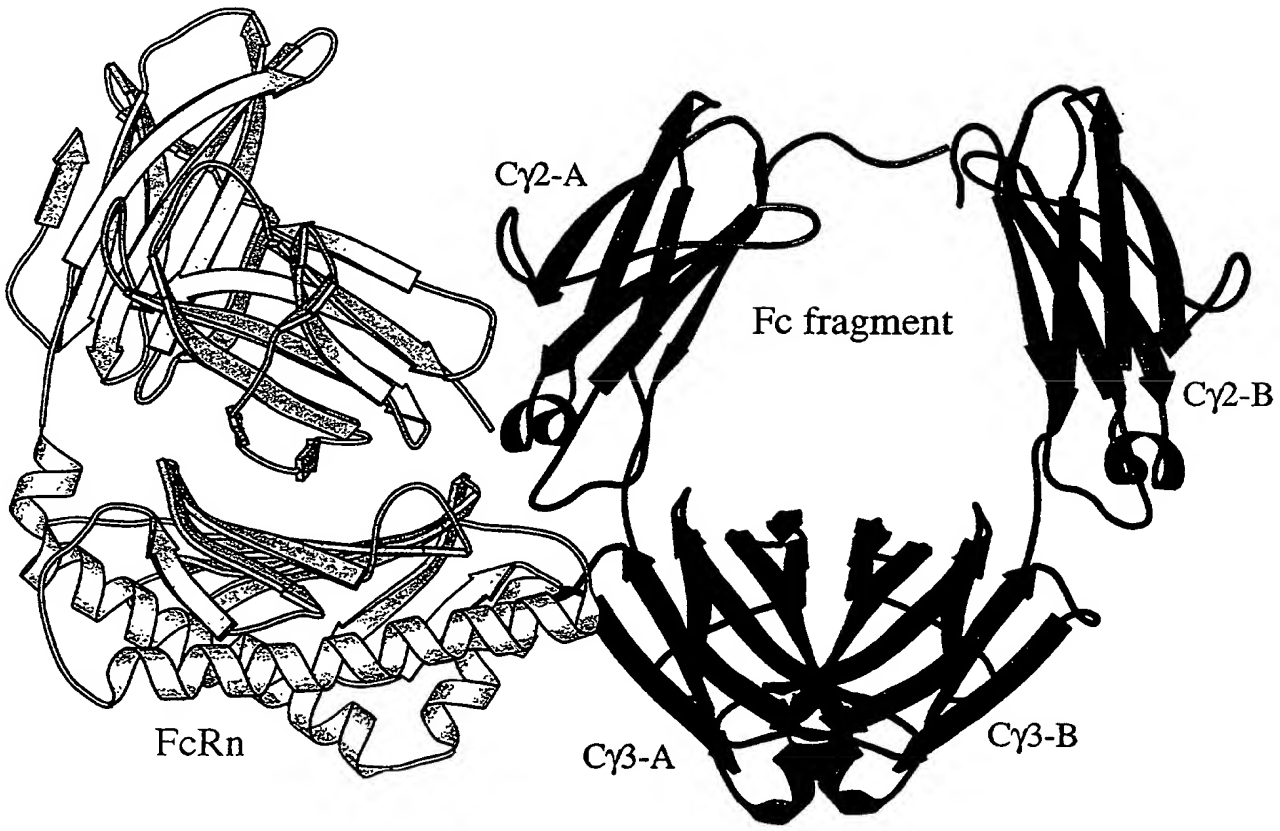


Fig. 2

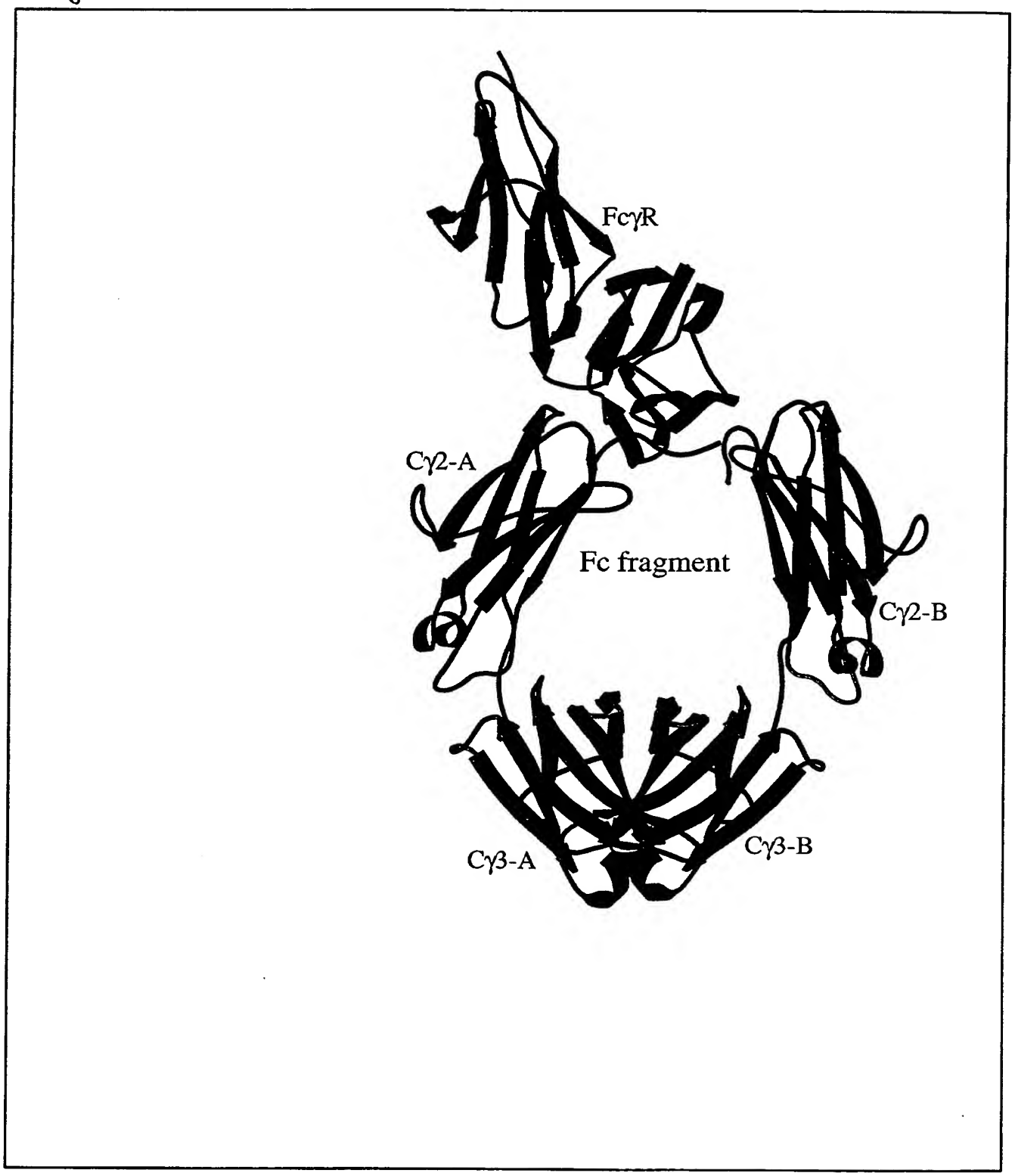
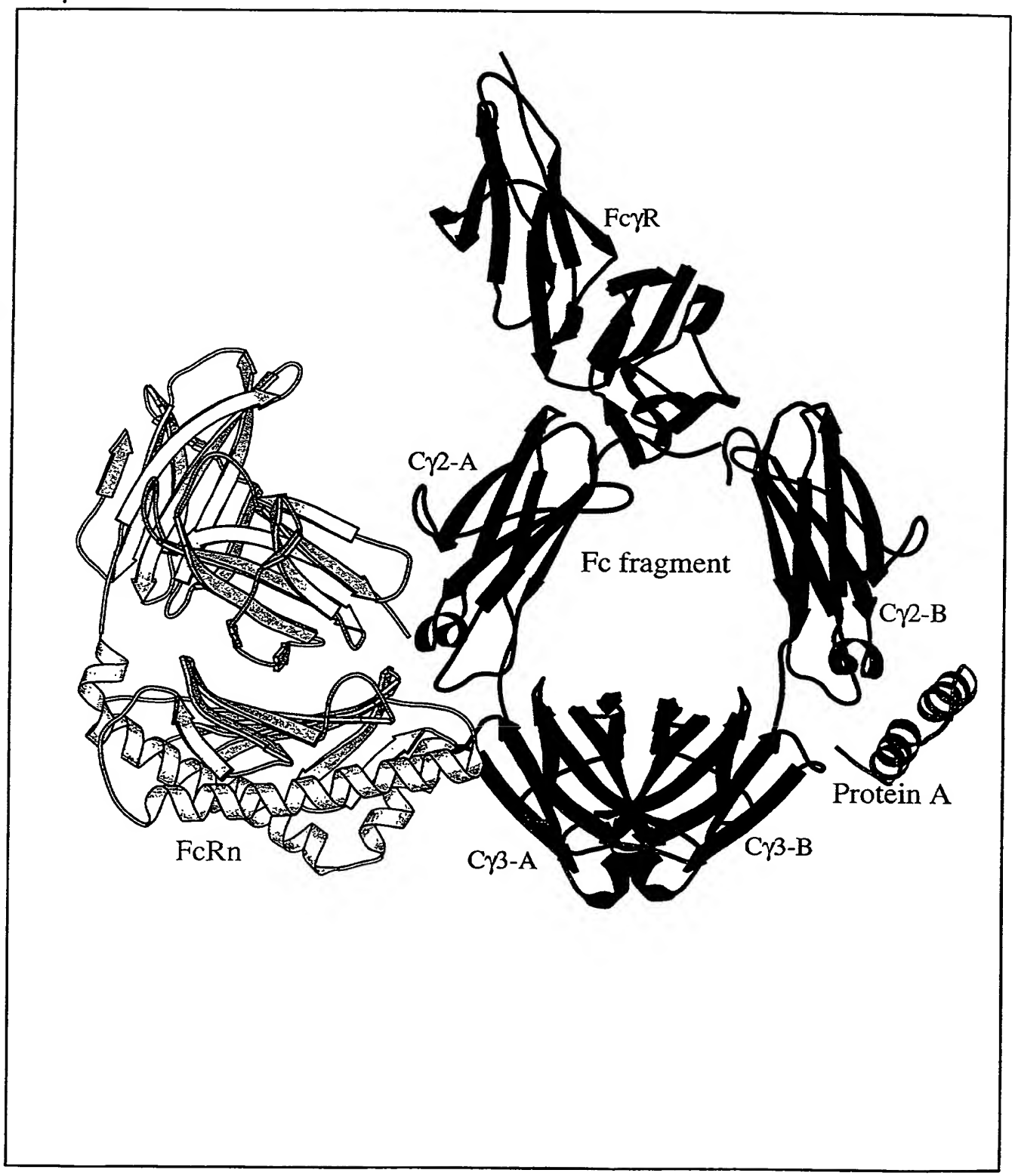


Fig. 3



PAT T COOPERATION TREATY

From the INTERNATIONAL BUREAU

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

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in its capacity as elected Office

Date of mailing (day/month/year) 14 August 2000 (14.08.00)	
International application No. PCT/EP99/09440	**Applicant's or agent's file reference** 19290P WO
International filing date (day/month/year) 03 December 1999 (03.12.99)	**Priority date** (day/month/year) 03 December 1998 (03.12.98)
Applicant SONDERMANN, Peter et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

19 June 2000 (19.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A1

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Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: RECOMBINANT SOLUBLE Fc RECEPTORS

(57) Abstract

Recombinant soluble Fc receptors according to the present invention are characterized by the absence of transmembrane domains, signal peptides and glycosylation. Such Fc receptors can easily be obtained by expressing respective nucleic acids in prokaryotic host cells and renaturation of the obtained inclusion bodies, which procedure leads to a very homogenous and pure product. The products can be used for diagnostic as well as pharmaceutical applications and also for the generation of crystal structure data. Such crystal structure data can be used for the modelling of artificial molecules. A further embodiment comprises coupling the Fc receptors according to the invention to solid materials like chromatography materials that can be used to separate and/or enrich antibodies.

FOR THE PURPOSES OF INFORMATION ONLY

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Recombinant soluble Fc receptors

Specification

The present invention relates to recombinant soluble Fc receptors (FcR), recombinant nucleic acids coding for such Fc receptors, host cells containing corresponding nucleic acids as well as a process for the determination of the amount of antibodies of a certain type contained in the blood, plasma or serum of a patient, a process for the determination of the immune status of patients with chronic diseases of the immune system and a process for the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors. Further, the present invention is concerned with pharmaceutical compositions containing the recombinant soluble FcRs, crystalline preparations of FcRs and FcR/Ig-complexes and especially of the use of such crystalline preparation for the generation of crystal structure data of Fc receptors as well as FcR inhibitors and pharmaceutical compositions containing such FcR inhibitors.

A still further subject of the present invention is a recombinant Fc receptor coupled to a solid phase, e.g. a chromatography carrier material. The use of such chromatography material, which is another subject of the present invention, lies in the absorption of immunoglobulins from a body fluid of patients or from culture supernatants of immunoglobulin producing cells.

Fc receptors (FcRs) play a key role in defending the human organism against infections. After pathogens have gained access to the blood circulation they are opsonized by immunoglobulins (Igs). The resulting immunocomplexes bind due to their multivalency with high avidity to FcR bearing cells leading to clustering of the FcRs, which triggers several effector functions (Metzger, H., 1992A). These include, depending on the expressed FcR type and

associated proteins, endocytosis with subsequent neutralization of the pathogens and antigen presentation, antibody-dependent cellular cytotoxity (ADCC), secretion of mediators or the regulation of antibody production (Fridman et al, 1992; van de Winkel and Capel, 1993).

Specific FcRs exist for all Ig classes, the ones for IgG being the most abundant with the widest diversity. Together with the high affinity receptor for IgE (FcϵRIa), FcγRI (CD64), FcγRII (CD32) and FcγRIIIa (CD16) occur as type I transmembrane proteins or in soluble forms (sFcRs) but also a glycosylphosphatidylinositol anchored form of the FcγRIII (FcγRIIIb) exists. Furthermore, FcγRs occur in various isoforms (FcγRIa, b1, b2, c; FcγRIIa1-2, b1-3, c) and alleles (FcγRIIa1-HR, -LR; FcγRIIIb-NA1,-NA2) (van de Winkel and Capel, 1993). In contrast to the overall homologous extracellular parts, the membrane spanning and the cytoplasmic domains differ. They may be deleted entirely or be of a size of 8 kDa. They may contain either a 26 amino acid immunoreceptor tyrosine-based activation motif (ITAM) as in FcγRIIa or a respective 13 amino acid inhibitory motif (ITIM) in FcγRIIb involved in signal transduction (Amigorena et al, 1992).

Judged by the conserved spacing of cysteins, the extracellular part of the FcRs consists of three (FcγRI, CD64) or two (FcϵRI, FcγRII, CD32 and FcγRIII, CD16) Ig-like domains (10 kDa/domain) and therefore belongs to the immunoglobulin super family. These highly glycosylated receptors are homologues, and the overall identity in amino acid sequence among the FcγRs and FcϵRIa exceeds 50% in their extracellular regions. Nevertheless, the affinity of FcRs to their ligands varies widely. The higher affinity of $\approx 10^8 M^{-1}$ of the FcγRI to Fc-fragment is assigned to its third domain, while the other FcγRs with two domains have an affinity to IgG varying between 10^5 and $10^7 M^{-1}$. The affinity of the two domain FcϵRIa to IgE exceeds these values by far with a constant of $10^{10} M^{-1}$ (Metzger, H., 1992B). In contrast to the mentioned FcRs the low affinity receptor for IgE FcϵRII represents a type transmembrane protein and shows a lower homology.

FcγRs are expressed in a defined pattern on all immunological active cells. FcγRI is constitutively expressed on monocytes and macrophages and can be induced on neutrophils and eosinophils. The physiological role of FcγRI is still unknown as the expression on monocytes is not vital (Ceuppens et al, 1988). The GPI anchored form of FcγRIII (FcγRIIIb) is exclusively expressed on granulocytes. Due to its missing cytoplasmic part, the signal transduction into the cell occurs solely via other transmembrane proteins like complement receptor type 3 (CR3) that can at least associate with FcγRIIIb (Zhou et al, 1993; Poo et al, 1995). FcγRIIIa is mainly expressed on monocytes and macrophages but only in conjunction with associated proteins (e.g. α- or γ-chains). FcγRII is the receptor with the widest distribution on immunocompetent cells and is mainly involved in the endocytosis of immunocomplexes.

FcγRIIa and FcγRIIb differ in their extracellular region by only 7% of the amino acid residues. Nevertheless, both forms can be distinguished by their binding characteristics to human and mouse IgG subclasses (van de Winkel and Capel, 1993) and their differing affinity to human IgGs (Sondermann et al, 1998A). The situation is rendered even more complicated by the high responder/low responder (HR/LR) polymorphism of FcγRIIa named after the ability of T cells from some individuals to respond to murine IgG1-induced mitogenesis (Tax et al, 1983). Later, it was found that the two exchanges in the amino acid sequence between the LR and the HR form modify the ability to bind human IgG2, which leads to the suggestion that at least one of them is involved in IgG binding (Hogarth et al, 1992).

In contrast to the beneficial role FcRs play in the healthy individual, they also transmit the stimulation of the immune system in allergies (FcεRIa) or autoimmune diseases. Moreover, some viruses employ FcγRs to get access to cells like HIV (Homsy et al, 1989) and Dengue (Littaua et al, 1990) or slow down the immune response by blocking FcγRs as in the case of Ebola (Yang et al, 1998) and Measles (Ravanel et al, 1997).

Hence, the object underlying the present invention was to provide receptors which are easy to produce and can advantageously be used for medical or diagnostic applications. Moreover, it was an object of the invention to provide soluble receptors exhibiting a binding specificity and activity which is analogous to that of the receptors occurring naturally in the human body and which, additionally, make it possible to produce crystals suitable for a structure determination.

This object is accomplished by recombinant soluble Fc receptors which consist only of the extracellular portion of the receptor and are not glycosylated. The receptors according to the present invention are therefore characterized by the absence of transmembrane domains, signal peptides and glycosylation.

Particularly preferred for the present invention are Fcγ or Fcϵ receptors. This is because IgG and IgE molecules are characteristic for a multiplicity of diseases and conditions, so that their determination and possible ways of influencing them are of great interest. Figure 11 and 12 show an alignment of amino acid sequences of the extracellular parts of some FcγRs and FcϵRI. The FcRs according to the invention include all these sequences or parts thereof that still retain binding capacity to antibodies and/or proper crystallization.

In a particularly preferred embodiment of the invention the recombinant soluble FcR is a FcγRIIb receptor. Further, it is particularly preferred that the receptor be of human origin. In a particularly preferred embodiment, it contains an amino acid sequence as shown in one of SEQ ID NO:1 to SEQ ID NO:6.

According to the present invention, the preparation of the soluble Fc receptors preferably takes place in prokaryotic cells. After such expression, insoluble inclusion bodies containing the recombinant protein form in

prokaryotic cells, thus facilitating purification by separation of the inclusion bodies from other cell components before renaturation of the proteins contained therein takes place. The renaturation of the FcRs according to the present invention which are contained in the inclusion bodies can principally take place according to known methods. The advantage of the preparation in prokaryotic cells, the production of inclusion bodies and the thus obtained recombinant soluble Fc receptors make it possible to obtain a very pure and, in particular, also very homogeneous FcR preparation. Also because of the absence of glycosylation the obtained product is of great homogeneity.

Soluble Fc receptors hitherto produced by recombinant means particularly exhibited the disadvantage that a much more elaborate purification was required, since they were expressed in eukaryotic cells and, due to the glycosylation which is not always uniform in eukaryotic cells, these products were also less homogeneous.

The recombinant soluble Fc receptors according to the present invention even make it possible to produce crystals suitable for use in X-ray analysis, as shall be explained lateron in the description of further embodiments of the invention. The FcRs of the present invention moreover exhibit practically the same activity and specificity as the receptors naturally occurring in vivo.

A further subject matter of the present invention is a recombinant nucleic acid having a sequence coding for a recombinant soluble Fc receptor according to the present invention.

The nucleic acid according to the present invention may contain only the coding sequences or, additionally, vector sequences and/or, in particular, expression control sequences operatively linked to the sequence encoding the recombinant FcR, like promoters, operators and the like.

In a particularly preferred embodiment the nucleic acid of the present invention contains a sequence as shown in one of SEQ ID NO:7 to SEQ ID NO:12. For a comparison, SEQ ID NO:13 and SEQ ID NO:14 show the respective wild type sequences coding for FcγRIIb and FcεRIa. SEQ ID NOs:15-18 show the wild type sequences for FcγRI, FcγRIIa, FcγRIII and FcεRII.

If the nucleic acid of the present invention contains vector sequences, then these are preferably sequences of one or several prokaryotic expression vectors, preferably of pET vectors. Any other known functions or components of expression vectors may also be contained in the recombinant nucleic acid according to the present invention if desired. These may, for instance, be resistance genes allowing for an effective selection of transformed host cells.

A still further subject matter of the present invention is a host cell containing a recombinant nucleic acid according to the present invention. As repeatedly mentioned above, the host cell preferably is a prokaryotic host cell, particularly an E. coli cell.

The recombinant soluble Fc receptors according to the present invention can be used for a multitude of examinations or applications because they specifically react with antibodies. In vivo, the soluble Fc receptors are powerful immunoregulators which, if present in elevated levels, result in a remarkable suppression of the immune system which leads to many partly known and partly not yet understood effects. Based on these effects, several applications of the Fc receptors according to the present invention are further subject matters of the present invention.

One such subject is a process for the determination of the amount of antibodies of a certain type in the blood or serum of a patient, which is characterized by the use of a recombinant soluble FcR according to the

invention in an immunoassay, and the determination of the presence of FcR-antibody complexes. Such assay allows to screen for the presence of a certain kind of antibody and allows also for the determination of the amount of antibodies present in the blood, plasma or serum of a patient.

Any type of immunoassay is principally suitable for the use according to the present invention, as long as the presence of FcR-antibody complexes can thereby be detected. Both ELISA (enzyme-linked immunosorbent immunoassay), particularly sandwich assays, and RIA (radio-immunoassay) are suitable, but also competitive testing methods. In a preferred embodiment of the invention where the presence and/or the amount of IgE antibodies is to be examined, an FcϵR is used as recombinant soluble receptor according to the present invention. In particular, this method is suited and advantageous for determining a predisposition or manifestation of an allergy.

Moreover, a method is preferred in which the presence of soluble FcRs is to be determined and, if required, quantified. For such determination preferably a competitive immunoassay method is used, wherein as competition reagent a recombinant soluble receptor according to the invention is used, most preferably a recombinant FcγR. By means of this test among others the immune status of patients with chronic diseases of the immune system can be determined in a competitive immunoassay. Chronic diseases in the sense of these processes are for instance AIDS, SLE (systemic lupus erythematosus), MM (multiple myeloma) or rheumatoid arthritis, or in the case of FcϵRII in B-CLL (Gordon et al., 1987), hyper IgE syndrome (Sarfati et al., 1988) or HCL (Small et al., 1990).

A further advantageous use of the recombinant receptor according to the present invention lies in the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors.

By means of modern screening techniques such as HTPS (high throughput screening) in combination with multi-well microtiter plates and automatic pipetting apparatuses it is nowadays possible to simultaneously test a multitude of substances for specific properties. As the FcRs according to the present invention can be easily produced at low cost, they can also be used in such series tests by which substances having an inhibiting effect can easily be identified.

Particularly preferred is such use according to which Fc receptors according to the present invention are used to find or screen inhibitors capable of inhibiting the recognition and binding of the respective antibodies to the particular receptor of interest.

A further area of application of the substances according to the invention lies in the pharmaceutical field. Hence, a further subject matter of the invention is a pharmaceutical composition comprising as active agent a recombinant soluble FcR according to the invention. According to the present invention, this pharmaceutical composition may of course comprise conventional useful carrier and auxiliary substances. Such substances are known to the person of skill in the art, the mode of administration also having to be taken into account. The pharmaceutical composition of the present invention can be advantageously used for the treatment or prevention of autoimmune diseases, allergies or tumor diseases.

Soluble forms of Fc receptors such as FcγRIII mediate isotype-specific regulation of B cell growth and immunoglobulin production. In a murine model of myeloma, sFcR suppresses growth and immunoglobulin production of tumor cells (Müller et al, 1985; Roman et al, 1988; Teillaud et al, 1990). Furthermore, sFcR binds to surface IgG on cultures of human IgG-secreting myeloma cells and effects suppression of tumor cell growth and IgG secretion. Prolonged exposure of these cells to sFcR results in tumor cell cytolysis (Hoover et al, 1995).

Also, overreactions of the immune system in allergic reactions or due to massive antigen load might be reduced by, for example, intravenous application of soluble FcR (Ierino et al, 1993).

Therefore, a preferred pharmaceutical composition according to the invention for use in the treatment of AIDS, rheumatoid arthritis or multiple myeloma contains a recombinant soluble Fcγ receptor and, preferably, a receptor having the amino acid sequence as shown in SEQ ID NO:1-4.

It was also of great interest to obtain crystal structure data of Fc receptors and/or Fc receptor/Ig complexes. On the one hand, these are a key to the understanding of molecular mechanisms in immunocomplex recognition. On the other hand, these structural data can be used to find out common features in the structures of different Fc receptors and use the knowledge of the structures to generate inhibitors or identify and produce new artificial antibody receptors.

It was also of great interest to obtain information on the concrete binding sites of immunoglobulins to their respective receptors in naturally occuring three-dimensional molecules. Therefrom even more precise findings on the interactions between antibody and receptor can be obtained and also on how these interactions can be modulated. In this connection modulation means either an enhancement of the interaction or a reduction leading to an inhibition by e.g. covering the binding sites on one or more parts of the complex.

To obtain such crystal structure data and conformation information, a crystalline preparation of the recombinant soluble Fc receptor according to the invention is used. The recombinant soluble FcRs according to the invention surprisingly can be obtained pure enough to produce crystals that give reliable X-ray structure determination data. Such crystallization was not

possible with the hitherto produced receptor molecules, mostly due to their lack of homogeneity.

Therefore, another embodiment of the present invention concerns a crytalline preparation of an Fc receptor according to the invention. Yet another embodiment of the present invention is a crystalline preparation of a complex of soluble Fc receptor according to the invention together with the related immunoglobulin Fc part. Particulary preferred embodiments are shown in the examples as well as the relevant crystal structure data. Via crystal structure analysis of the crystalline preparations the exact amino acids of the Fc receptor/Ig complexes could be detected which mediate the coupling. These amino acids are in shown Fig 6a and 6b and the type of binding between the individual amino acids of both molecules in the complex is also indicated. A further embodiment of the present invention is therefore the use of a crystalline preparation of a recombinant soluble Fc receptor for the generation of crystal structure data of Fc receptors. From this crystal structure data information about the three-dimensional structure and the active sites for the binding of antibodies can be obtained. Especially preferably is the use of a crystalline preparation of a complex of recombinant soluble Fc receptor according to the invention and the corresponding immunoglobulin molecule for the generation of crystal structure data for the complexes. These data allow to determine the actual interactions that are formed between the two molecules and allow for the first time to obtain exact information about the interaction of the molecules thereby conferring knowledge about possible sites for inhibition or enhancement of the binding. On the basis of the information obtained from the crystal structure data the findings necessary for effecting modulation of the interaction between Fc receptor and immunoglobulin can be obtained. This modulation can be range from enhancement to complete inhibition to an inhibition of the binding.

The stated applications are merely preferred embodiments of the use of the crystal structure data. Many other applications seem possible, too.

Suitably, the structural data for the generation and/or identification of inhibitors or new receptors, respectively, are used in a computer-aided modelling program.

Particularly preferred for the present invention are the structures of FcRs or FcR:Fc-fragment complexes as examplified in figures and examples. Such structures can be used to design inhibitors, antagonists and artificial receptor molecules.

Computer programs suitable for computer-aided drug design and screening are known to the person skilled in the art and generally available. They provide the possibility to examine umpteen compositions on the computer in view of their ability to bind to a certain molecule when the corresponding structure dates are entered in the computer. With the help of this possibility a great number of known chemical compositions can be examined regarding their inhibiting or antagonistic effect. The person skilled in the art merely requires the crystal structure dates provided by the present invention and a commercially available screening program (Program Flexx: From the GMD-German National Research Center for Information Technology, Schloss Birlinghoven, D-53754 Sankt Augustin, Germany). A preferred embodiment of the present invention therefore is the use of the crystal structure data obtained for the recombinant soluble Fc receptor according to the invention and for the complexes of recombinant soluble Fc receptor according to the invention and corresponding immunoglobulin in a computer aided modelling program for the identification and production of Fc receptor inhibitors.

Likewise, a further embodiment of the present invention is the use of the crystal structure data obtained for the receptors according to the invention and the receptor/immunoglobulin complexes, respectively for the

identification and preparation of new Fc receptors which can be used, e.g. as antagonists and competitors. The crystal structure data and the data on the amino acids involved in the binding to Fc receptors obtained therefrom can serve for example to generate mutated immunoglobulins which can also be used as inhibitors. It is imaginable that mutated or chemically modified inhibitors undergo tight binding and thus effect a blocking of receptors. On the other hand, the data obtained for the binding sites of immunoglobulins can also be used for the identification and/or preparation of inhibitors for immunoglobulin molecules. Since the present invention teaches the binding sites to the receptor, it is easy to effect a blocking of the binding sites with the help of relatively simple molecules. Therefore, a further subject matter of the present invention is the use of the crystal structure data obtained for the FcR/Ig complexes for the identification and/or preparation of immunoglobulin inhibitors.

Accordingly, still further subject matter of the present invention are FcR inhibitors which have a three-dimensional structure which is complementary to the recombinant soluble FcR according to the invention and inhibit the binding of antibodies to FcRs.

Another further subject of the present invention are immunoglobulin inhibitors which have a three-dimensional structure which is complementary to the immunoglobulin binding site for recombinant soluble Fc receptors according to the invention and inhibit the binding of immunoglobulins to Fc receptors.

The term "complementary" is to be understood within the framework of the invention in such a way that the inhibitor molecules must be substances which are able to cover at least so many binding sites on the immunoglobulin or on the Fc receptor that the binding between Fc receptor and immunoglobulin is at least decisively weakened. Covering can take place both by binding to the amino acids mediating the complex formation

of either component but also in such a way that at least complex formation is no longer possible, be it by sterically inhibition or by binding to adjacent amino acids, however, covering the amino acid involved in the complex binding between Fc receptor and immunoglobulin.

In connection with the present invention it was possible for the first time to determine the exact binding sites and the amino acids involved in the binding of the antibody and antibody receptor molecules. One is now able to design specifically binding molecules and to screen candidate compositions on the computer. This enables the selection of such compositions from a variety of possibly candidate compositions which can effect a sufficient inhibition of complex formation between Fc receptor and immunoglobulin.

What is important for the inhibitors of the invention is that, owing to their structure and specificity, they are capable of binding to the FcRs or immunoglobulins and thus prevent the normal binding between FcRs and the constant parts of antibodies.

Preferably, such FcR or IgG inhibitors are small organic molecules which can easily be administered orally. They are an interesting alternative to cortisone in the treatment of autoimmune diseases and host/graft rejections. Such a molecule would also suppress reinfection rates with certain viruses, e.g. Dengue virus where the antibody coated virus is FcγRIIb dependent internalized (Littaua et al, 1990), HIV where on CD4 positive T cells an antibody enhancement of HIV infection is mediated by FcγRIII (Homsy et al, 1989), or Ebola where the virus secreted glycoprotein inhibits early neutrophil activation by blocking sFcγRIII which affects the host response to infection (Yang et al, 1998).

The development of inhibitors also leads to substances that interfere with the recognition of IgE by their receptors. From the modelled structure of

FcεRI, peptides have already been developed which inhibit mast cell degranulation in vitro. With the now available knowledge of the structures of the homologue receptors and the receptor-antibody complex in atomic detail, a new possibility for a rational drug design is opened.

The Fc-receptor bind between the two CH2-domains of the Fc-fragment in the so-called lower hinge region (Fig.8). The binding region of the Fc-receptor is described in Example 1 (The contact interface to IgG). The residues promoting the interaction between FcR and immunoglobulin are shown in figures 7, 10a and 10b. Thereby three interaction regions become evident (Fig.5).

1st region: FcR (residues 85 to 87 and residue 110) - Ig (Chain A residues 326-328)

Proline 328 of the Ig is clamped by the residues Trp 87 and 110 in a sandwich like manner. These residues are conserved among the IgG and IgE receptors as well as in the IgG and IgE. An inhibitor binding to this prominent region would strongly interfere with binding. This region is additionally attractive for inhibitor design because the exposed hydrophobic surface region comprising the residues Trp 87, Ile 85, Gly 86 of the receptors could be employed to obtain additional binding energy. The functional groups of Thr 113 and Glu 18 and Lys 19 side chains in the vicinity may contribute especially to specific inhibitor binding.

2nd region: FcR (residues 126-132 and residues 155-158) - Ig (Chain A and Chain B residues 234-239)

The amino terminal residues 234-239 of both Ig chains are recognised differently by the FcR, thereby breaking the 2-fold symmetry of the Fc fragment.

This residues of Fc-fragment chain A are in contact with residues Val 155 - Lys 158 of the receptor and the same residues from Fc-fragment chain B with receptor residues Gly 126 - His 132. This region shows the most

differences in the sequence alignment of the receptors as well as the immnoglobulins and should therefore be involved in specificity generation. This deep cleft between the Fc-fragment chains is well suited for inhibitor design and would be the site of choice for the development of inhibitors when issues of specificity are concerned.

3rd region: FcR (residues 117, 126 and 129-132) - Ig (Chain B residues 264-265 and residues 296-297)

This binding region is characterised by a clustering of amino acid residues carrying functional groups in their side chains, that might be employed in various ways for inhibitor design on the receptor and the Ig side of the contact.

Molecules that interact with one or more of the above described regions, and are designed or screened explicitly for exploiting the knowledge of binding sites are considered as inhibitors according to the invention.

Further subject matters of the present invention are pharmaceutical compositions containing as active agent an FcR inhibitor or an immunoglobulin inhibitor as mentioned above. Such pharmaceutical compositions may, for example, be used in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system, preferably the treatment or prevention of allergies, autoimmune diseases or anaphylactic shock.

A further subject of the present invention is the sFcR according to the invention, bound to a solid phase. Such heterogeneous receptors can be used for immunoassays or other applications where the receptor in an immobilized form can be used beneficially.

In a preferred embodiment of the invention the solid phase is a chromatography carrier material onto which the Fc receptor is fixed, e.g.

sepharose, dextransulfate etc. Such chromatography materials with Fc receptors bound thereto can beneficially be used for the adsorption of immunoglobulins from the blood, plasma or serum of patients or from the culture supernatant of immunoglobulin producing cells (meaning concentration, enrichment and purification of antibodies).

On the one hand, the antibodies bound to the chromatography material can be eluted and, for example, the immune status of a patient can thereby be determined. On the other hand, antibodies from the blood of a patient can thereby be enriched before carrying out further tests, which is a further preferred embodiment of the present invention. In many cases it is difficult to conduct diagnostic assays using blood samples if the latter contains only a very small number of the antibodies to be identified. By means of a concentration using a specific chromatographic column with Fc receptors according to the present invention, antibodies of interest can easily be concentrated and separated from many other substances which might disturb the test.

Basically, it is also possible to use a chromatography material according to the present invention in an extracorporeal perfusion system for lavage of the blood in case of certain diseases where the removal of antibodies plays a crucial role.

It is, however, also possible to use another material as solid phase to which the soluble Fc receptor according to the invention is coupled, e.g. microtiter plates or small reaction vessels to the walls of which Fc receptors are bound either directly or indirectly. Such solid phases and vessels can be particularly important for diagnostic methods, as they enable screening by using immunoassays e.g. for detecting the presence of certain immunoglobins in patients' blood or other body fluids.

To sum up, the recombinant soluble Fc receptors provided by the present invention as well as the corresponding structure determination of crystalline

preparations of these receptors and of crystalline complexes of receptors and immunoglobins enable for the first time to perform a rational drug design, wherefrom it is possible to modulate the interaction between immunoglobulins and Fc receptors on cells or soluble receptors. Such a modulation is preferably an inhibition, whereby the inhibition of the formation of a complex from IgG and Fc receptor takes place by covering and preferably by binding of inhibitor molecules to the Fc receptor or the immunoglobulin. There are various medical applications for such modulating drugs and in particular of inhibitors and only few of these applications have been exemplary mentioned within the framework of the present specification. This can and should by no means exclude the applicability of such molecules which have been designed or screened on the basis of the findings about the molecular structure or FcR/Ig complexes disclosed herein for the treatment or prevention of other health disturbances.

The following Examples are to further illustrate the invention in conjunction with the Figures.

Example 1

shFcγRIIb (soluble human FcγRIIb)

1.1 Cloning and Expression

The cDNA of human FcγRIIb2 (Engelhardt et al, 1990) was modified using mutagenous PCR (Dulau et al, 1989). Therefore, a forward primer was used for the introduction of a new start methionine after the cleavage site of the signal peptide within a *Nco*I site (5'-AAT AGA ATT CCA TGG GGA CAC CTG CAG CTC CC-3') while the reverse primer introduced a stop codon between the putative extracellular part and the transmembrane region followed by a *Sal*I site (5' CCC AGT GTC GAC AGC CTA AAT GAT CCC C-3'). The PCR product was digested with *Nco*I and *Sal*I, cloned into a pET11d expression vector (Novagen) and the proposed sequence was

confirmed. The final construct was propagated in BL21(DE3) (Grodberg and Dunn, 1988). For the overexpression of FcγRIIb a single colony of the transformed bacteria was inoculated in 5ml LB medium containing 100 µg ampicillin per ml (LB-Amp100) and incubated overnight at 37°C. The culture was diluted 200-fold in LB-Amp100 and incubation was continued until an OD600 of 0.7-0.9 was achieved. The overproduction of the protein was induced by adding IPTG to a final concentration of 1 mM. After a growing period of 4 hours the cells were harvested by centrifugation (30 min, 4000 x g) and resuspended in sonification buffer (30 mM sodium phosphate, 300 mM sodium chloride, 0.02% sodium azide, pH 7.8). After addition of 0.1 mg lysozyme per ml suspension and incubation for 30 min at room temperature the sonification was performed on ice (Branson Sonifier, Danbury, CT; Macrotip, 90% output, 80% interval, 15 min). The suspension was centrifuged (30 min, 30,000 x g) and resuspended with a Dounce homogenizer in sonification buffer containing 0.5% LDAO. The centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The purified inclusion bodies were stored at 4°C.

1.2 Refolding and purification of soluble human FcγRIIb (shFcγRIIb)

The purified inclusion bodies were dissolved to a protein concentration of 10 mg/ml in 6 M guanidine chloride, 100 mM 2-mercaptoethanol and separated from the insoluble matter by centrifugation. The refolding was achieved by rapid dilution. Therefore, one ml of the inclusion body solution was dropped under stirring within 15 hours into 400 ml of the refolding buffer (0.1 M TRIS/HCl, 1.4 M arginine, 150 mM sodium chloride, 5 mM GSH, 0.5 mM GSSG, 0.1 mM PMSF, 0.02% sodium azide, pH 8.5, 4°C). Afterwards, the mixture was stirred for 2-3 days until the concentration of free thiol groups was reduced to 1 mM by air oxidation as measured according to Ellman (Ellman, 1959). The solution was dialyzed against PBS and sterile filtered before it was concentrated 10-fold in a stirring cell

equipped with a 3kD MWCO ultrafiltration membrane. The protein solution was applied to a hIgG sepharose column (50 mg hIgG per ml sepharose 4B). Unbound protein was washed out with 50 mM TRIS pH 8.0 before elution of FcγRIIb by pH jump (150 mM sodium chloride, 100 mM glycine, 0.02% sodium azide, pH 3.0). The eluate was immediately neutralized with 1 M TRIS pH 8.0. The FcγRIIb containing solution was concentrated and subjected to gel filtration on a Superdex-75 column equilibrated with crystallization buffer (2 mM MOPS 150 mM sodium chloride, 0.02% sodium azide pH 7.0). The fractions containing FcγRIIb were pooled, concentrated to 7 mg/ml and stored at -20°C.

1.3 Equilibrium gel filtration experiments

A Superdex75 column was connected to FPLC and equilibrated with PBS containing 10 µg shFcRIIb per ml. Human Fc fragment was solved to a concentration of 1 µg/10 µl in the equilibration buffer and injected. The resulting chromatogram yielded a positive peak comprising the complex of the shFcγRIIb and the Fc fragment while the negative peak represents the lack of receptor consumed from the running buffer for complex formation.

1.4 Crystallization and data collection

Initial crystallization trials employing a 96 condition sparse matrix screen (Jancarik and Kim, 1991) were performed in sitting drops at 20 °C using the vapor diffusion method. Occuring crystals were improved by changing the pH as well as the salt, precipitant and additive concentration. Diffraction data from suitable crystals was collected on an image plate system (MAR research) using graphite monochromated CuK_α radiation from a RU200b rotating anode generator (Rigaku) operated at 50 kV and 100 mA. The reflections were integrated with the program MOSFLM (Leslie, 1997) and subsequently the data was scaled, reduced and truncated to obtain the

structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

1.5 Summary of expression, purification and refolding of shFcγRIIb

The extracellular part of FcγRIIb was expressed in high levels under the control of a T7 promoter in the T7 RNA polymerase positive E. coli strand BL21/DE3 (Grodberg & Dunn, 1988). The protein was deposited in inclusion bodies, which were employed in the first purification step. The isolation of the inclusion bodies was started with an intense combined lysozyme/ sonification procedure to open virtually all cells which would otherwise contaminate the product. The subsequent washing steps with the detergent LDAO, which has excellent properties in solving impurities but not the inclusion bodies itself already yielded a product with a purity of >90% (Fig. 1).

This product was used for refolding trials without further purification. The inclusion bodies were dissolved in high concentration of 2-mercaptoethanol and guanidine to ensure the shift of covalent and non-covalent aggregates to monomers. This solution was rapidly diluted with refolding buffer to minimize contacts between the unfolded protein molecules which would otherwise form aggregates. The use of arginine in the refolding buffer prevents the irreversible modification of side chains as often recognized with urea. After addition of the protein to the refolding buffer, the solution was stirred at 4°C until the concentration of free thiol groups was reduced to 1 mM, which was absolutely necessary as earlier dialysis resulted in an inactive product. In a second purification step the dialyzed and refolded FcγRIIb was bound to immobilized hIgG to remove minor fractions of E. coli proteins and inactive receptor. The protein was eluted with a pH jump and immediately neutralized. After this affinity chromatography step shFcγRIIb is essentially pure except for a minor contamination resulting from the coeluting IgG which leached out of the matrix even after repeated use (Fig.

1). The IgG as well as receptor multimers which are not visible in the reducing SDS-PAGE could easily be removed by gel filtration. Parallel to the removal of the contaminants in this step the buffer is quantitatively exchanged. This procedure ensures a defined composition of the protein solution as even slight variations can cause irreproducibility of the crystallization attempts or even inhibit the formation of crystals. Overall 6 mg pure protein could be gained per litre E. coli culture, which is about 10 % from the FcγRIIb content of the inclusion bodies.

N-terminal protein sequencing revealed the identity with the expected sequence H_2N-GTPAAP without detectable contamination. ESI-MS analysis showed that the final material used in crystallization trials is homogenous with respect to size. From the primary sequence the molecular weight was calculated to 20434 Da, which corresponds to 20429 Da found by mass spectroscopy. The discrepancy lies within the error of the instrument, and no additional peak for a species containing the leading methionine is found.

The crystallization of shFcγRIIb was performed in sitting drops using the vapor diffusion method. Initial trials with a sparse matrix screen (Jancarik & Kim, 1991) resulted already in small crystalline needles. Subsequent optimization of the preliminary crystallization condition by varying precipitant, salt, their concentration and pH led to the isolation of three different crystal forms. Orthorhombic crystals grew from mixture of 1.5 μl reservoir solution (33% PEG2000, 0.2 M sodium acetate, pH 5.4) with 3 μl of the protein solution. They appeared within 3 days and reached their final size of approximately 80 μm x 80 μm x 500 μm after one week. These crystals diffracted to 1.7 Å. Crystals could also be grown in two other space groups from reservoir solution containing 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 5 mM $Zn(OAc)_2$, 100 mM sodium chloride (hexagonal form) and 26% PEG8000, 0.2 M NaOAc, pH 5.6, 10% (v/v) 1,4-Dioxan, 100 mM sodium chloride (tetragonal form). These crystals were

of suitable size for X-ray analysis but diffracted only to 2.7 Å and 3.8 Å for the tetragonal and hexagonal crystal form respectively (Table 1).

FcγRII was expressed in E. coli which, besides the comparatively low production costs and the availability, has several advantages especially when the glycosylation performed by mammalian cells is not necessary for the function of the protein as in the case of FcγRII where IgG binding occurs independently of carbohydrate attachment (Sondermann et al, 1998A). In E. coli a homogenous product can reproducibly be generated, which is in contrast to the expression in mammalian cells where batch dependent variances are often observed. In such a system the product is for several days exposed to proteases at temperatures of more than 30°C. In contrary, the expression of the protein in E. coli under the control of the strong T7 promoter at 37°C frequently leads to the formation of protease inaccessible inclusion bodies. A further advantage of the expression in bacteria is that the material could be considered to be free of pathogenic germs, which might derive from employed fetal calf serum or the cell line itself. In mammalian expression particular care must be taken during the purification of the target protein because potential effective hormones or growth factors might be copurified. One case where the effects of sFcγR were ascribed to a TGFβ1 contamination is already reported (Galon et al, 1995).

1.6 Purification

The purification procedure is straightforward. It consists of three steps which can easily be performed in a single day. The protein is obtained in a pure form and in high yields and could even be obtained in considerable quality without the expensive IgG affinity column. The success of such a protocol would depend on the careful preparation of the inclusion bodies, as most of the impurities can be eliminated already in the first purification step.

1.7 Characterization

The purified FcγRIIb was characterized by SDS-PAGE and isoelectric focussing as well as N-terminal sequencing and mass spectroscopy. Thus, the material can be considered pure and homogeneous with respect to its chemical composition, but the intriguing question whether the receptor is correctly folded remains to be discussed. All cysteins are paired, since no free thiol groups are detected with Ellman's test. The material is monomeric and eludes with the expected retention time in peaks of symmetrical shape from a size exclusion chromatography column. Furthermore, FcγRIIb binds to IgG sepharose, recombinant FcγRIIb from E. coli is active because it specifically binds IgG.

1.8 Crystallization

The orthorhombic crystal form of FcγRIIb diffracted X-rays to a resolution of 1.7 Å, which is a drastic improvement compared to previously reported crystals of the same molecule derived from insect cell expression (Sondermann et al, 1998A). These crystals diffracted to 2.9 Å and were of space group $P3_121$. Thus, the glycosylation of the insect cell derived receptor influences the crystallization conditions. Instead of the trigonal space group, three different crystal forms are found. After a possible solution of the structure these crystal forms will help identify artificial conformations of the protein due to crystal contacts.

FcγRs do not exhibit any sequence similarity to other proteins but due to a conserved cystein spacing they are affiliated to the immunoglobulin super family. Consequently, we tried to solve its structure by molecular replacement, but extensive trials using IgG domains from a variety of molecules failed. Thus the structure of FcγRIIb has to be solved by the methods of multiple isomorphous replacement.

We have shown for the first time that FcγRIIb can be obtained in an active form from E. coli. This is the basis for crystallographic investigations that will soon, due to the already gained crystals of exceptional quality, result in the structure solution of this important molecule. The structure will provide information on the IgG binding site and provide a starting point for the knowledge based design of drugs that interfere with recognition of the ligand by its receptor. Furthermore, because of the high homology between FcγRIIb and other FcRs including FcεRIa it seems possible that these molecules can be produced in the same way, which would provide valuable material for the ongoing research.

1.9 Methods

Protein chemistry

Recombinant soluble human FcγRIIb was expressed in E.coli, refolded purified and crystallized as described elsewhere (Sondermann et al, 1998B). Briefly, the putative extracellular region of hFcγRIIb2 (Engelhardt et al, 1990) was overexpressed in E. coli. Inclusion bodies were purified by lysozyme treatment of the cells and subsequent sonification. The resulting suspension was centrifuged (30 min 30,000 x g) and washed with buffer containing 0.5% LDAO. A centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The inclusion bodies were solved in 6 M guanidine hydrochloride and the protein was renaturated as described. The dialyzed and filtrated protein solution was applied to a hIgG sepharose column and eluted by pH jump. The concentrated neutralized fractions were subjected to size-exclusion chromatography on a Superdex-75 column (26/60, Pharmacia).

Crystallization

Crystallization was performed in sitting drops at 20°C using the vapor diffusion technique. Crystallization screens were performed by changing pH,

salt, precipitant and additives. The final crystals used for data collection were grown in 33% PEG2000, 0.2 M sodium acetate, pH 5.4 (orthorhombic form) 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 10% (v/v) 1,4-dioxane, 100 mM sodium chloride (tetragonal form), and 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 5mM $ZN(OAc)_2$, 100 mM sodium chloride (hexagonal form). The insect cell derived protein was crystallized in 32% PEG6000, 0.2 M sodium acetate, pH 5.3.

Preparation of heavy-atom derivatives

The heavy-atom derivatives were prepared by soaking the crystals in the crystallization buffer containing 2 mM platinum(II)-(2,2'-6,2''terpyridinium) chloride for 24 hours or 10 mM uranylchloride for 8 days.

X-ray data collection

Diffraction data was collected on an image plate system (MAR research) using graphite monochromated CuK_α radiation from a RU200b rotating anode generator (Rigaku) operated at 50 kV and 100 mA. The reflections were integrated with the program MOSFLM 5.50 (Leslie, 1997) and subsequently the data was scaled and truncated to obtain the structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

Structure determination

The structure was solved with the standard procedures of the MIR method. From the large number of soaks carried out with different heavy-atom components only the two compounds yielded interpretable Patterson maps. The heavy-atom positions for each derivative were determined from difference Patterson maps and initial phases were calculated. Cross-phased difference Fourier maps were used to confirm heavy atom positions and establish a common origin for the derivatives. Anomalous data were included to discriminate between the enantiomers. The heavy atom parameters were further refined with the program MLPHARE from the CCP4

package leading to the statistics compiled in Table 2. An electron-density map was calculated to a resolution of 2.1 Å and the phases were improved further by solvent flattening and histogram matching with the program DM from the CCP4 suite. The resulting electron density map was of sufficient quality to build most of the amino acid residues. Model building was performed with O (Jones et al, 1991) on an Indigo2 work station (Silicon Graphics Incorporation). The structure refinement was done with XPLOR (Brünger et al, 1987) by gradually increasing the resolution to 1.7 Å using the parameter set of Engh and Huber (Engh & Huber, 1991). When the structure was complete after several rounds of model building and individual restraint B-factors refinement (R_{fac} = 29% / R_{Free} = 36%), 150 water molecules were built into the electron density when a Fo-Fc map contoured at 3.5 σ coincided with well defined electron density of a 2Fo-Fc map contoured at 1 σ. The resulting refinement statistic is shown in Table 3.

1.10 Structure determination

The crystal structure of recombinant soluble human FcγRIIb was solved by multiple isomorphous replacement (MIR) to 1.7 Å resolution, since a structure solution by molecular replacement with isolated domains of the Fc fragment from human IgG1 (Huber et al, 1976, PDB entry 1fc1; Deisenhofer, 1981) failed. The putative extracellular part of the receptor (amino acid residues 1-187 as depicted in SEQ ID NO:2) was used for crystallization trials (Sondermann et al, 1998B) while the model contains the residues 5-176 as the termini are flexible and not traceable into the electron density. Additionally, the model contains 150 water molecules and the refinement statistics are summarized in Table 2. The structure contains a cis proline at position 11. None of the main chain torsion angles is located in disallowed regions of the Ramachandran plot. The fully refined model was used to solve the structure of the same protein in crystals of space group $P4_22_12$ and of the glycosylated form derived from insect cells in crystals of space group $P3_121$ (Table 2).

The polypeptide chain of FcγRIIb folds into two Ig-like domains as expected from its affiliation with the immunoglobulin super family. Each domain consists of two beta sheets that are arranged in a sandwich with the conserved disulfide bridge connecting strands B and F on the opposing sheets (Fig. 3). Three anti-parallel β-strands (A1, B, E) oppose a sheet of 5 β-strands (C', C, F, G, A2), whereby strand A1 leaves the 3-stranded β-sheet and crosses over to the 4-stranded anti-parallel sheet adding the short parallel 5th strand A2. The arrangement of secondary structure elements as well as their connectivity is identical in both domains of the FcγRIIb and a rigid body fit of one domain onto the other revealed a r.m.s. distance of 1.29 Å of 67 matching Cα atoms.

The domains are arranged nearly perpendicularly to each other enclosing an angle of 70 degrees between their long axes forming a heart-shaped overall structure. This arrangement results in an extensive contact region between the domains (Fig. 4). Residues from strand A2 and from the segment linking A2 and A1 of the N-terminal domain intermesh with residues of strands A1 and B from the C-terminal domain. This region is tightly packed and the interaction is strengthened by several hydrogen bonds resulting in a rigid arrangement. This is confirmed by the conservation of the structure in three different space groups. In orthorhombic, tetragonal and hexagonal (insect cell derived) crystal forms a deviation of less than 2° in the interdomain angle is found.

1.11 Overall structures

The structure of recombinant human FcγRIIb derived from E.coli was solved by MIR to 1.7 Å resolution from orthorhombic crystals. An essentially identical structure is found in tetragonal and with protein derived from insect cells in hexagonal crystals. In all three structures the last nine residues of the polypeptide chain were found disordered. The flexibility of the C-terminal linker region between the structured core of the molecule and

the transmembrane part may be functionally relevant to allow some reorientation of the receptor to enhance the recognition of the Fc parts in immunocomplexes.

1.12 Homologue receptors

The Ig domains found in the Ig super family of proteins are characterized by a beta sandwich structure with a conserved disulfide bridge connecting two strands of the opposing sheets. The typical arrangement of 3 and 4 anti parallel beta strands that form a sandwich as found in FcγRIIb occurs also in the T cell receptor, Fc fragment, CD4 or the Fab fragment. A structural alignment of the individual Ig domains of these molecules with the two domains of FcγRIIb shows a common, closely related structure. The relative arrangement of the domains, however, is not related in these molecules and covers a broad sector. Despite the structural similarity between Ig domains from different molecules and the strikingly low r.m.s. deviation of Cα atoms that result when the two domains of FcγRII are superimposed, no significant sequence similarity is found (Figs. 5a and 5b). A structure-based sequence alignment shows a conserved hydrophobicity pattern along the sequence of the domains, together with, beside the cysteins, only few identical amino acid residues. We first prepared a structure-based alignment of the two C-terminal domains of the IgG1 heavy chain and the FcγRIIb and added the sequences of the other related FcγR and the FcεRIa domains. This shows that the sequences of the three domain FcγRI and the two domain receptors are compatible with the hydrophobicity pattern of Ig domains and several conserved amino acid residues are revealed. Firstly, the different domains of an FcR are more related to each other than to Ig domains from other molecules of the Ig super family. Secondly, the N-terminal domains of the receptors relate to each other as the second domains do. Thirdly, the sequence of the third domain of FcγRI shows features from both groups of domains. Taken together, we confirm the affiliation of the FcRs to the Ig super family and speculate that all FcR-domains originate from a common

ancestor, an ancient one domain receptor that acquired a second domain by gene duplication. Further divergent development of such a two domain receptor resulted in the present diversity, including FcγRI that acquired a third domain.

Conservation of these amino acid residues that contribute to the interdomain contact in FcγRIIb in the alignment are a hint to a similar domain arrangement in different receptors. In Table 4 the residues contributing with their side chains to the interdomain contact (Fig. 4) are compiled for FcγRIIb together with the corresponding amino acid residues in other receptors according to the structure-based sequence alignment of Fig. 5b. Except for Asn15, which is not conserved between the FcRs, the involved residues are identical or conservatively replaced providing strong support for a similar structure and domain arrangement in all FcRs.

1.13 The contact interface to IgG

Limited information about the interactions of FcRs with their ligands is available from mutagenesis studies (Hogarth et al, 1992; Hulett et al, 1994; Hulett et al, 1995). By systematically exchanging loops between the β-strands of FcγRIIa for FcεRIa amino acid residues the B/C, C'/E and F/G loops of the C-terminal domain were evaluated as important for ligand binding (Fig. 3, Fig. 5b). In the structure model these loops are adjacent and freely accessible to the potential ligand. Additionally, most of the amino acid residues in these loops were exchanged for alanines by single site mutations which resulted in a drastic alteration of the affinity of FcγRIIa to dimeric human IgG1. Also, the single amino acid exchange Arg 131 to His in the C-terminal domain (C'/E loop) in the high responder/low responder polymorphism, which alters the affinity of the FcγRIIa to murine IgG1, points to that region. Thus, the amino acid residues in this area are either important for ligand binding or the structural integrity of that region. Here, the structure shows a clustering of the hydrophobic amino acid residues Pro

114, Leu 115 and Val 116 in the neighbourhood of Tyr 157. This patch is separated from the region Leu 159, Phe 121 and Phe 129 by the positively charged amino acid residues Arg 131 and Lys 117 which protrude from the core structure (Fig. 5b).

1.14 Glycosylation

In the sequence of FcγRIIb three potential N-glycosylation sites are found. All three sites are on the surface of the molecule and are accessible. They are located in the E/F loops (N61 and N142) of both domains and on strand E (N135) of the C-terminal domain (Fig. 3, Fig. 6). Since the material used for the solution of this structure was obtained from E. coli, it does not contain carbohydrates, while the FcRs isolated from mammalian cells are highly glycosylated. The three potential glycosylation sites are located rather far from the putative IgG binding region, and non-glycosylated FcγRIIb binds human IgG, suggesting a minor role of glycosylation in binding. This was confirmed by the structure of the FcγRIIb produced in insect cells which is glycosylated (Sondermann et al, 1998A). Except for a 2° change of the interdomain angle possibly due to different crystal contacts, no differences between the glycosylated and unglycosylated protein structures were found. The three glycosylation sites are only optionally used as shown by SDS-PAGE where the material appears in 4 bands. No additional electron density for those sugars was found a consequence of chemical and structural heterogeneity.

Example 2

shFcγRIIa (soluble human FcγRIIa)

The procedures were performed according to example 1 except for the indicated changes:

2.1 Cloning and Expression

shFcγRIIa was generated by mutating the respective wild-type cDNA (Stengelin et al., 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET22b + vector was chosen.

2.2 Refolding and purification

shFcγRIIa was refolded according to example 1 with the respective refolding buffer listed in table 6.

2.3 Crystallisation

shFcγRIIa was crystallised as described under conditions indicated in table 7.

2.4 Structure determination

The structure was solved with the method of isomorphous replacement with shFcγRIIb as search model.

Example 3

shFcγRIII (soluble human FcγRIII)

The procedure was performed according to example 1 except for the indicated changes:

3.1 Cloning and Expression

shFcγRIII was generated by mutating the respective wild-type cDNA (Simmons & Seed, 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET22b + vector was chosen.

3.2 Refolding and purification

shFcγRIII was refolded according to example 1 with the respective refolding buffer listed in table 6.

3.3 Crystallisation

shFcγRIII was crystallised as described under conditions indicated in table 7.

3.4 Structure determination

The structure was solved with the method of isomorphous replacement with shFcγRIIb as search model.

3.5 Crystallisation of a shFcγRIII:hFc1 complex

hIgG1 derived from the serum of a myeloma patient was used to prepare Fc-fragments (hFc1) by digestion with plasmin (Deisenhofer et al., 1976). The resulting Fc-fragments were separated from the Fab-fragments by protein A chromatography. Partially digested hIgG was removed by size exclusion chromatography with MBS (2mM MOPS, 150mM NaCl, 0.02% sodium azide, pH 7.0) as running buffer. Equimolar amounts of hFc1 and shFcgRIII were mixed and diluted with MBS to a concentration of 10mg/ml. The complex was crystallised as described under conditions indicated in table 5.

Example 4

shFcεRII (soluble human FcεRII)

The procedure was performed according to example 1 except for the indicated changes:

4.1 Cloning and Expression

FcεRII was generated by mutating the respective wild-type cDNA (Kikutani et al., 1986) and expressed according to example 2 with the mutagenous

primers listed in table 5. For the expression of the protein a pET23a+ vector was chosen.

4.2 Refolding and purification

Refolding of shFcϵRII was achieved as described in example 1, with the exception that prior to rapid dilution the dissolved inclusion bodies were dialysed against 6M guanidine chloride, 20mM sodium acetate, pH 4.0. shFcϵRII was refolded according to example 1 with the respective refolding buffer listed in table 6. After refolding the protein solution was dialysed against PBS, concentrated 100-fold and purified by gel filtration chromatography on Superdex 75. This yielded pure shFcϵRII which was dialysed against 2mM TRIS/HCl, 150mM NaCl, 0.02% sodium azide, pH 8.0, concentrated to 10mg/ml and stored at 4°C.

Example 5

shFcγRI (soluble human FcγRI)

The procedure was performed according to example 1 except for the indicated changes:

5.1 Cloning and Expression

shFcγRI was generated by mutating the respective wild-type cDNA (Allen & Seed, 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET32a+ vector was chosen, which contains after the N-terminal thioredoxin a hexahistidine-tag with a C-terminal thrombin cleavage site followed by the shFcγRI in frame with the mentioned proteins and amino acid residues. For the overexpression of the fusion protein the E.coli strain BL21(DE3) containing the plasmids pUBS and pLysS (Novagen) was used.

The purified inclusion bodies were solubilised in 6M guanidine-HCl, 10mM β-mercaptoethanol, 50mM Tris pH8.0 and bound to a Ni-NTA column

(Qiagen). The elution was performed with an imidazole gradient ranging from 0 to 1M imidazole. The eluted protein was dialysed against a 1000fold volume of 150mM NaCl, 50mM Tris pH8.0, 2mM GSH, 0.5mM GSSG for 24 hours at 4°C. After concentrating the protein solution to 25% of the initial volume, thrombin was added. After 6h of incubation at 37°C the N-terminal thioredoxin and the His-tag were removed completely as verified by N-terminal sequencing. During this digestion the shFcgRI precipitated quantitatively out of solution.

5.2 Refolding and purification

shFcγRI was refolded according to example 1 with the respective refolding buffer listed in table 6. After the redox potential decresased to 1mM the solution was dialysed against PBS pH8.0 and concentrated.
The refolded Protein was analysed by size exclusion chromatography, which yielded a peak of the proposed monomeric receptor and non reducing SDS-PAGE which showed a major band at 30kDa.

Example 6

shFcϵRIa (soluble human FcϵRIa)

The procedure was performed according to example 1 except for the indicated changes:

6.1 Cloning and Expression

shFcϵRI was generated by mutating the respective wild-type cDNA (Kochan et al., 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET23a+ vector was chosen.

Brief description of the figures

Fig. 1: 15% reducing SDS PAGE showing the purification of sFcγRIIb

Lane 1: Molecular weight marker. Lane 2: E. coli lysate before induction. Lane 3: E. coli lysate 1 h after induction. Lane 4: E.coli lysate 4 h after induction. Lane 5: Purified inclusion bodies of sFcγRIIb. Lane 6: Eluate of the hIgG affinity column. Lane 7: Pooled fractions of the gel filtration column.

Fig. 2: Equilibrium gel filtration

1 μg hFc solved in 10 μl equilibration buffer (10 μg sFcγRIIb/ml PBS) was applied to a size exclusion chromatography column and the absorbance of the effluent was measured (280 nm) as a function of time. The injected Fc fragment forms a complex with the sFcγRIIb in the equilibration buffer (t = 22min). The negative peak of consumed sFcγRIIb is observed at t = 26 min.

Fig. 3: Overall structure of human sFcγRIIb

Stereo ribbon representation of the sFcγRIIb structure. The loops supposed to be important for IgG binding are depicted in red with some of the residues within the binding site and the conserved disulfide bridge in ball and stick representation. The potential N-glycosylation sites are shown as green balls. The termini are labeled and the β-strands are numbered consecutively for the N-terminal domain in black and for the C-terminal domain in blue. The figure was created using the programs MOLSCRIPT (Kraulis, 1991) and RENDER (Merritt and Murphy, 1994).

Fig. 4: Interdomain contacts

The figure shows a close-up on the residues involved in the interdomain contacts of sFcγRIIb. The amino acid residues of the N-terminal domain are depicted blue and the residues of the C-terminal domain yellow. The model is covered by a 2Fo-Fc electron density contoured at 1 σ obtained from the

final coordinates. Hydrogen bridges between the domains are represented by white lines. The figure was created using the program MAIN (Turk, 1992).

Fig. 5a: Superposition of the two FcγRIIb domains and the CH2 domain of human IgG1

Both domains of FcγRIIb and the CH2 domain of hIgG1 were superimposed. The N-terminal domain is depicted in blue, the C-terminal domain in red and the CH2 domain of hIgG1 in green. The respective termini are labeled and the conserved disulfide bridges are depicted as thin lines.

Fig. 5b: Structure based sequence alignment of the sFcγFIIb domains with domains of other members of the FcR family

The upper part of the figure shows the structure based sequence alignment of the FcγRIIb and hIgG1 Fc fragment domains performed with the program GBF-3D-FIT (Lessel & Schomburg, 1994). Amino acid residues with a Cα distance of less than 2.0 Å in the superimposed domains are masked: lilac for matching residues between the Fc fragment domains; yellow for residues in the FcγRIIb domains; and green when they can be superimposed in all four domains. The β-strands are indicated below this part of the alignment and are labeled consistent with Figure 3.

The lower part of the figure shows the alignment of the amino acid sequences from the other FcγRs and the homologue FcεRIa to the profile given in the upper part of the figure using routines from the GCG package (Genetics Computer Group, 1994). The upper and lower row of numbering refer to the N- and C-terminal domains of FcγRIIb. The conserved cysteins are typed in magenta and the potential glycosylation sites in blue. Identical residues within the first domain are masked orange, those in the second domain pink and green when the residues are conserved within both domains. The less conserved third domain of FcγRI is aligned between the first and the second domains. Red arrows point to residues that are involved

in side chain contacts between the first and the second domain while blue arrows depict residues that are relevant for IgG binding. The figure was produced with the program ALSCRIPT (Barton, 1993).

Fig. 6: The putative binding sites of FcγRIIb

Solid surface representations of FcγRIIb as produced with GRASP (Nicholls et al, 1991), the color coding is according to the relative surface potential from negative (red) to positive (blue). Fig. 6a shows the molecule as in Fig. 3 by a rotation of about 90° counter-clockwise around the vertical. In Fig. 6b the molecule is rotated 90° clockwise around the same axis. Both views show the putative binding regions on the C-terminal (Fig. 6a) and the N-terminal domain (Fig. 6b). The amino acid residues discussed in the text are labeled.

Fig. 7: Cα-trace of the superpositioned structures of the Fcγ-receptors

FcγRIII red, FcγRIIa green and FcγRIIb blue. Residues important for IgG binding are shown in ball-and-stick. The N- and C-termini are labelled.

Fig. 8: Overview of the FcγRIII/Fc-fragment crystal structure in ribbon representation

The sugar residues bound to the Fc-Fragment are indicated in ball-and-stick. The FcγRIII (blue) binds in the lower hinge region between chain-B (red) and chain-A (green) of the Fc-fragment.

Fig. 9: Close-up on the binding region of the FcγRIII and the Fc-fragment

The colour scheme is in agreement to figure 8 and residues important for complex formation are shown in ball-and-stick.

Fig. 10a:

In the upper part of figure 10a a structure based sequence alignment of the Fc-Receptor ecto-domains is shown. Conserved residues are shaded yellow and identical residues orange. The lower part of the figure shows a part of

the alignment of human antibody sequences. Residues of the human FcγRIII in contact with the Fc-fragment in the complex crystal structure are connected by lines (black for hydrophobic interaction, red for salt bridges and blue for hydrogenbridges). Residues from the Fc-receptor in contact with the A-chain of the Fc-fragment are connected with dashed lines and those in contact with the B-chain of the Fc-fragment with solid lines. Red, blue and black lines represent charged, polar and other contacts, respectively.

Fig. 10b:

In the upper part of figure 10b a structure based sequence alignment of the Fc-Receptor ecto-domains is shown. Conserved residues are shaded yellow and identical residues orange. Conserved residues within the less related Kir and FcA-Receptor sequences are shaded blue. The lower part of the figure shows a part of the alignment of human antibodies with the mouse IgE (mIgE) sequence. Residues of the human FcγRIII in contact with the Fc-fragment in the complex crystal structure are connected by lines (black for hydrophobic interaction, red for salt bridges and blue for hydrogenbonds). Residues from the Fc-receptor in contact with the A-chain of the Fc-fragment are connected with dashed lines and those in contact with the B-chain of the Fc-fragment with solid lines. Red, blue and black lines represent charged, polar and other contacts, respectively.

Fig. 11 and Fig. 12:

Fig. 11 and Fig. 12 show an alignment of the produced sFcγR, sFcεRIa and the short form of sFcεRII and the produced sFcγR and sFcεRIa without sFcεRII, respectively.

Table 1: Crystallographic results

The obtained preliminary crystallographic data are shown in this table.

	Orthorhombic	Tetragonal	Hexagonal
Space group	$P2_12_12_1$ [19]	$P4_22_12$ [94]	P3 [143]
Unit cell dimensions	a=40.8Å,b=50.9Å, c=80.5Å, α=90°, β=90°,γ=90°	a=85.7Å,b=85.7Å, c=63.4Å, α=90°, β=90°,γ=90°	a=80.9Å,b=80.9Å c=157.0Å, α=90°, β=90°,γ=90°
R_{merge}	5.8%	9.8%	13.6%
Resolution	1.7Å	2.7Å	3.8Å
Unique	18,040	6,616	7,210
Completeness	89.1%	97.1%	63.0%
Multiplicity	3.5	4.4	1.3
V_M, molecules per asymmetric unit, solvent content	2.09Å^3/Da, 1mol., 41% solvent	2.91Å/Da, 1 mol, 58% solvent	2.97Å/Da, 5 mol, 59% solvent

Table 2: Data collection statistics

Derivative	Space Group	No. of unique reflections	Multi-plicity	Resolution (Å)	Complete-ness (overall/ last shell) (%/%)	R_m (%)	No. of sites	Phasing power
NATI	$P2_12_12_1$.	18009	3.6	1.74	92.9/86.4	5.5		
NATI	$P4_22_12$	6615	4.5	2.70	97.1/94.3	10.1		
NATI-Baculo	P3,21	3545	2.5	3.0	93.0/98.9	14.4		
UOAc	$P2_12_12_1$.	7722	4.2	2.1	96.8/95.7	7.3	1	1.79
PtPy	$P2_12_12_1$.	5520	3.9	2.3	89.7/49.6	10.5	1	1.39

$R_m = \Sigma I/_h - </_h> I/\Sigma </_h>$

Phasing power: $<F_H>/E$, where $<F_H> = \Sigma(F_H^2/n)^{1/2}$ is the r.m.s. heavy atom structure amplitude.

$E = \Sigma[(F_{PHC}-F_{PH})^2/n]^{1/2}$ is the residual lack of closure error with F_{PH} being the structure factor amplitude and $F_{PHC} = |F_P + F_H|$ the calculated structure factor amplitude of the derivative.

Table 3: Refinement statistics

Resolution range (Å)	8.0 - 1.74 Å
No. of unique reflections ($F>0\sigma(F)$)	16252
R factor R_{free}*	19.4 27.9
No. of atoms per asymmetric unit protein solvent	 1371 150
Rms deviation from ideal geometry bond length (Å) bond angle (°)	 0.009 2.007
Average B factors (Å^2) protein main chain protein side chain solvent	 18.8 25.2 36.7
Rms deviation of bonded B factors (Å^2)	4.1

* R_{free}: 5% of the reflections were used as a reference data set and were not included in the refinement.

Table 4: Residues that contribute to the interdomain contact via side chains

FcγRIIb	FcγRIIa	FcγRIII	FcγRI	FcεRIa
Asn15	Asn	Ser	Ser	Arg
Asp20	Asp	Asp	Glu	Glu
Gln91	Gln	Gln	Gln	Gln
His108	His	His	His	His
Trp110	Trp	Trp	Trp	Trp

Table 5: Primers used for the amplification of the FcRs

Construct	5'-Primer	3'-Primer
sFcγRI	5'-CACCCATATGGCAGTGATCTCTTT-3'	5'-AGGACTCGAGACTAGACAGGAGTTGGTAAC-3'

sFcγRIIa	5'-ACAGTCATATGGCAGCTCCCC-3'	5'-AAAAAAAGCTTCAGGGCACTTGGAC-3'
sFcγRIIb	5'-AATTCCATGGGGACACCTGCAGCTCCC-3'	5'-CCCAGTGTCGACAGCCTAAATGATCCCC-3'
sFcγRIII	5'-AAAAAAACATATGCGGACTGAAG-3'	5'-AAAAAAGCTTAACCTTGAGTGATG-3'
sFcεRIa	5'-GATGGCCATATGGCAGTCCCTCAG-3'	5'-CAATGGATCCTAAAATTGTAGCCAG-3'
sFcεRII	5'-AAAAAAACATATGGAGTTGCAGG-3'	5'-TGGCTGGATCCATGCTCAAG-3'

Introduced restriction sites are underlined, start- and stop-codons are depicted as bold-italics

Table 6: Refolding Conditions for the FcRs

Construct	Buffer
sFcγRI	0.1M TRIS/HCl, 1.2M arginine, 150mM NaCl, 5mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.0
sFcγRIIa	0.1M TRIS/HCl, 1.4M arginine, 150mM NaCl, 2mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.0
sFcγRIIb	0.1M TRIS/HCl, 1.4M arginine, 150mM NaCl, 5mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.0
sFcγRIII	0.1M TRIS/HCl, 1.0M arginine, 150mM NaCl, 2mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.0
sFcεRII	0.1M TRIS/HCl, 0.8M arginine, 150mM NaCl, 5mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.3

Table 7: Crystallisation Conditions for the FcRs

Construct	Condition	Space group, cell constants	Resolution
sFcγRIIa	26% PEG 8000, 0.2M sodium acetate/acetic acid pH 4.6, 0.02% sodium azide	C2, a=80.4Å, b=49.7Å, c=54.6Å, a=g=90°, b=128.1°	3.0Å

sFcγRIIb	33% PEG 2000, 0.2M sodium acetate, 0.02% sodium azide, pH5.4	P212121, a=40.8Å, b=50.9Å, c=80.5Å, a=b=g=90°	1.7Å
sFcγRIII	22% PEG 8000, 0.1M MES/TRIS pH 7.8, 0.02% sodium azide	P22121, a=36.7Å, b=60.3Å, c=85.6Å, a=b=g=90°	2.5Å
sFcγRIII: hFc1	6% PEG 8000, 0.1M MES/TRIS pH 5.6, 0.2M Na/K tartrate, 0.02% sodium azide	P6522, a=b=115.0Å, c=303.3Å, a=b=90°, g=120°	3.3Å
sFcγRIII	22% PEG 8000, 0.1M MES/TRIS pH 7.8, 0.02% sodium azide	P22121, a=36.7Å, b=60.3Å, c=85.6Å, a=b=g=90°	2.5Å

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Claims

1. Recombinant soluble Fc receptor characterized by the absence of transmembrane domains, signal peptide and glycosylation.

2. Recombinant Fc receptor according to claim 1, wherein the receptor is a FcγR or a FcϵR.

3. Recombinant Fc receptor according to claim 1 or 2, wherein the receptor is a FcγRIIb.

4. Recombinant Fc receptor according to any one of claims 1 to 3, wherein the receptor is of human origin.

5. Recombinant Fc receptor according to any one of claims 1 to 4, wherein it contains one of the amino acids as shown in one of SEQ ID NOs:1-6.

6. Recombinant nucleic acid containing a sequence encoding a recombinant Fc receptor according to any one of claims 1 to 5.

7. Recombinant nucleic acid according to claim 6, wherein it contains one of the sequences as shown in one of SEQ ID NOs:7-12.

8. Recombinant nucleic acid according to claim 6 or 7, wherein it additionally contains expression control sequences operably linked to the sequence encoding the recombinant Fc receptor.

9. Recombinant nucleic acid according to any one of claims 6 to 8,

wherein it is contained on a prokaryotic expression vector, preferably a pET vector.

10. Host cell characterized by the presence of a recombinant nucleic acid according to any one of claims 6 to 8.

11. Host cell according to claim 10,
wherein it is a prokaryotic host cell, preferably an E. coli cell.

12. Process for the determination of the amount of antibodies of a certain type in the blood, plasma or serum of a patient, characterized by the use of a recombinant soluble Fc receptor according to any one of claims 1 to 5 in an immunoassay and determination of the presence of FcR-antibody complexes.

13. Process according to claim 12, wherein the immunoassay is an ELISA and preferably a sandwich assay.

14. Process according to claim 12 or 13, wherein the antibodies to be determined are IgE antibodies and the recombinant soluble receptor is a FcϵR.

15. Process according to claim 14 for the determination of a predisposition or manifestation of an allergy.

16. Process according to claim 12 or 13, wherein the antibodies to be determined are IgG antibodies and the recombinant soluble receptor is a FcγR.

17. Process for the determination of the immune status of patients with chronic diseases of the immune system, wherein a Fc receptor according to any one of claims 1 to 5 is used in a competitive

immunoassay and the amount of the corresponding sFcRs in the blood, plasma or serum of a patient is determined.

18. Process according to claim 17, wherein the chronic disease is AIDS, SLE, MM or rheumatoid arthritis.

19. Use of a recombinant soluble Fc receptor according to any one of claims 1 to 5 for the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors.

20. Use according to claim 19, wherein recombinant soluble FcγRs are used and recognition and binding of IgG antibodies is of interest.

21. Pharmaceutical composition containing as active agent a recombinant soluble FcR according to any one of claims 1 to 5.

22. Pharmaceutical composition according to claim 21 for use in the treatment or prevention of autoimmune diseases, allergies or tumor diseases.

23. Pharmaceutical composition according to claim 21 or 22 for use in the treatment of AIDS, rheumatoid arthritis or multiple myeloma, containing a recombinant soluble FcγR preferably having the amino acid sequence as shown in SEQ ID NO:1.

24. Crystalline preparation of a soluble recombinant Fc receptor according to claims 1 to 5.

25. Crystalline preparation of a soluble recombinant Fc receptor / immunoglobulin complex.

26. Use of a crystalline preparation of a recombinant soluble Fc receptor according to any one of claims 1 to 5 for the generation of crystal structure data of Fc receptors.

27. Use of a crystalline preparation of a soluble recombinant Fc receptor / immunoglobulin complex for the generation of crystal structure data of receptor / Ig complexes and their respective binding sites.

28. Use of crystal structure data obtained by the use according to claims 26 or 27 for the identification and/or preparation of Fc receptor or immunoglobulin inhibitors.

29. Use of crystal structure data obtained by the use according to claim 26 or 27 for the identification and preparation of new antibody receptors.

30. Use according to any one of claims 26 to 29 in a computer-aided modelling program.

31. FcR inhibitor characterized in that it has a three-dimensional structure which is complementary to the recombinant soluble FcR according to any one of claims 1 to 5.

32. Immunoglobulin-inhibitor, characterized in that it has a three-dimensional structure which is complementary to an Fc receptor binding site of an immunoglobulin.

33. Pharmaceutical composition containing as active agent a FcR inhibitor according to claim 31.

34. Pharmaceutical composition containing as active agent an immunoglobulin inhibitor according to claim 32.

35. Pharmaceutical composition according to claim 33 or 34 for use in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system.

36. Pharmaceutical composition according to claim 33, 34 or 35 for the treatment or prevention of allergies, autoimmune diseases or an anaphylactic shock.

37. Use of a molecule for the modulation of the interaction between Fc receptor and immunoglobulin, characterized in that
the molecule is designed or identified using crystal structure data obtained from crystalline preparations according to claims 24 or 25.

38. Use according to claim 37, wherein
the modulation is partial or complete inhibition of binding between Fc receptor and immunoglobulin.

39. Fc receptor according to claims 1-5, bound to a solid phase.

40. Fc receptor according to claim 39, wherein the solid phase is a chromatography carrier material.

41. Use of a chromatography carrier material according to claim 40 for the adsorption of immunoglobulins from the blood, plasma or serum of a patient or from culture supernatants of immunoglobulin producing cells.

42. Use according to claim 41 for the enrichment of antibodies from a patient's blood, serum or plasma or from culture supernatants of immunoglobulin producing cells for the conduction of further tests.

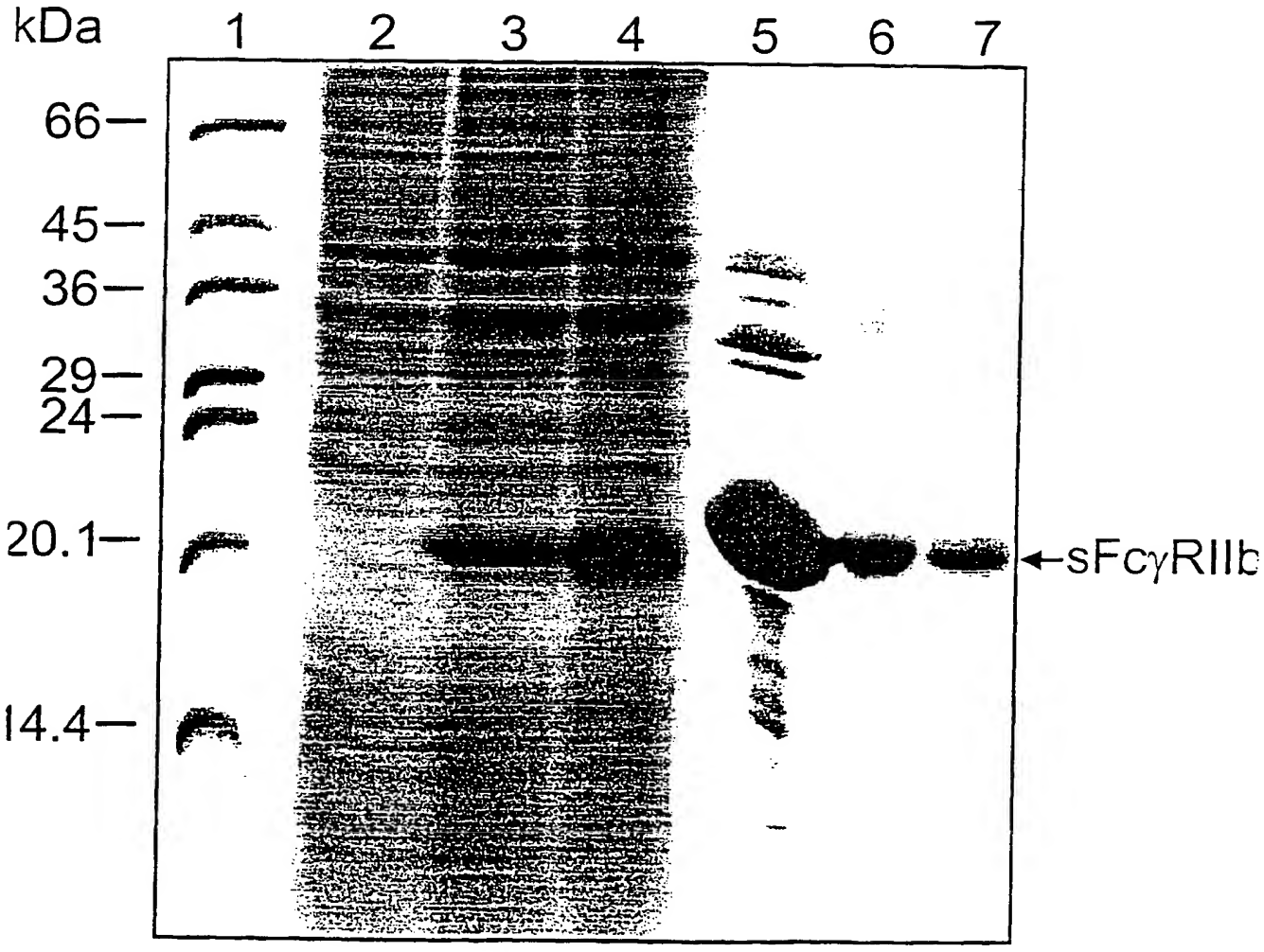

FIG. 1

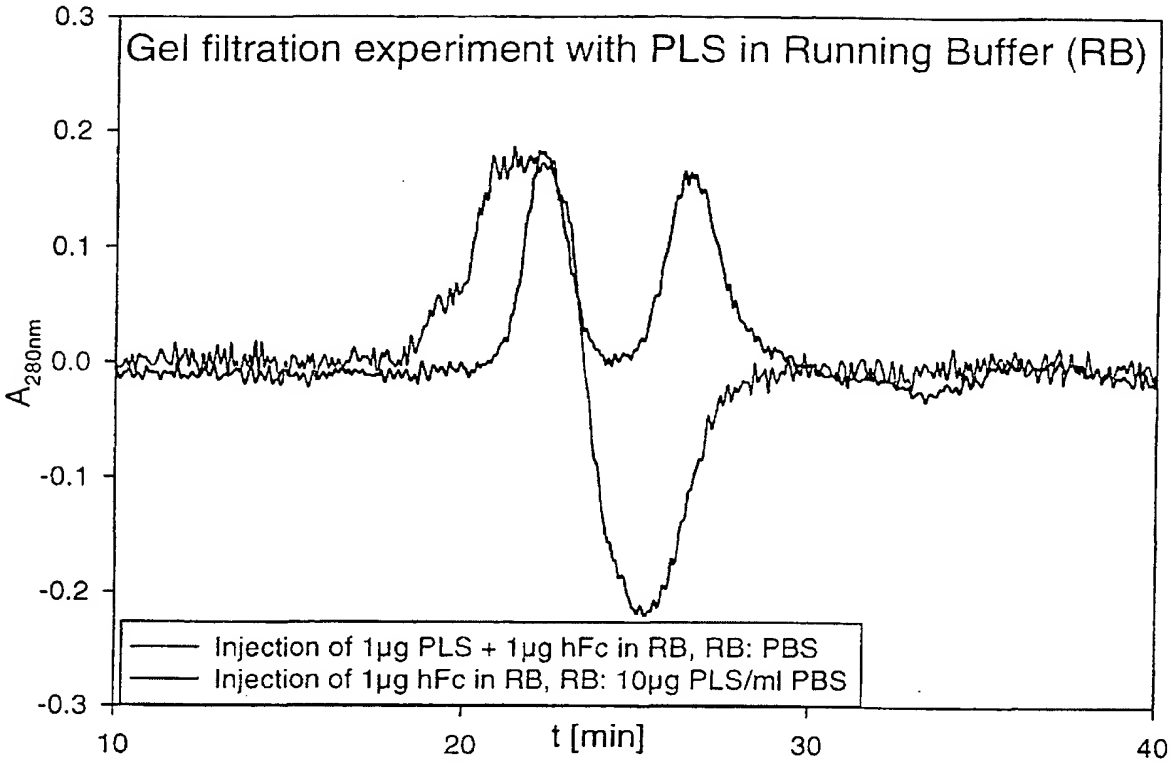
Gel filtration experiment with PLS in Running Buffer (RB)
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
A_{280nm}
10
20
30
40
t [min]
Injection of 1µg PLS + 1µg hFc in RB, RB: PBS
Injection of 1µg hFc in RB, RB: 10µg PLS/ml PBS

FIG. 2

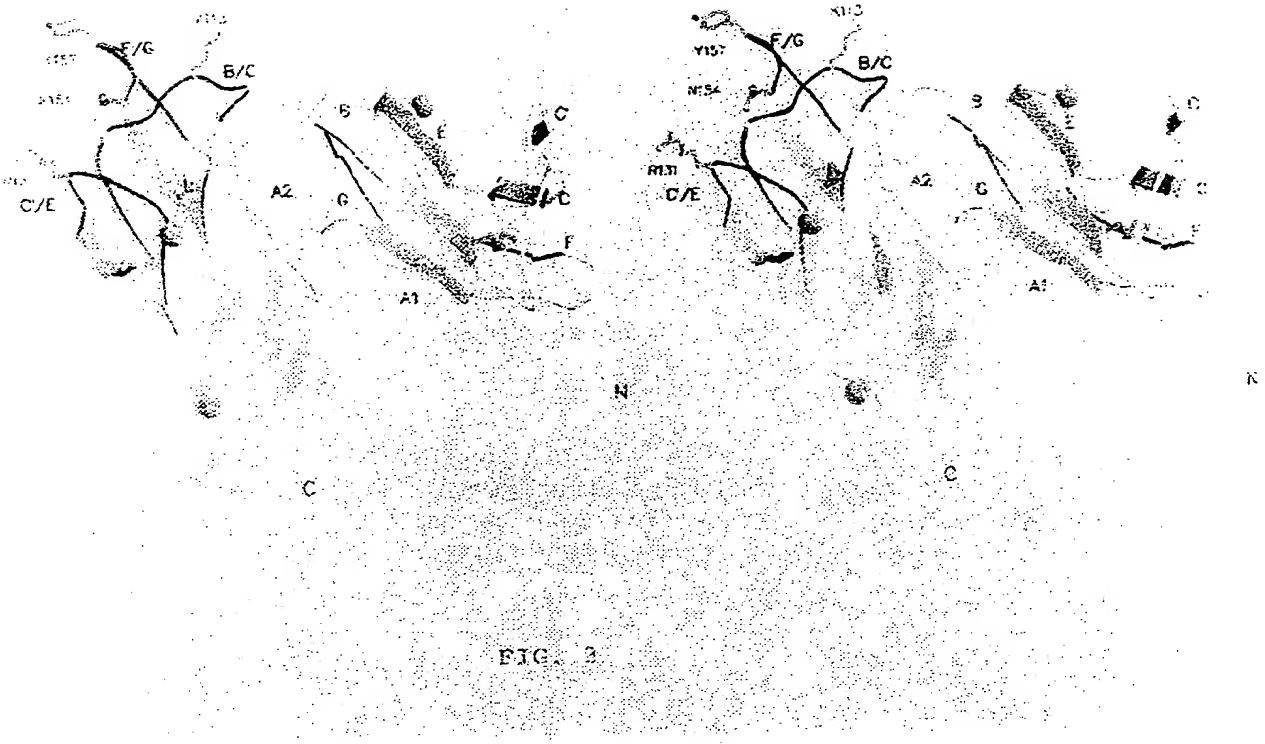

FIG. 3

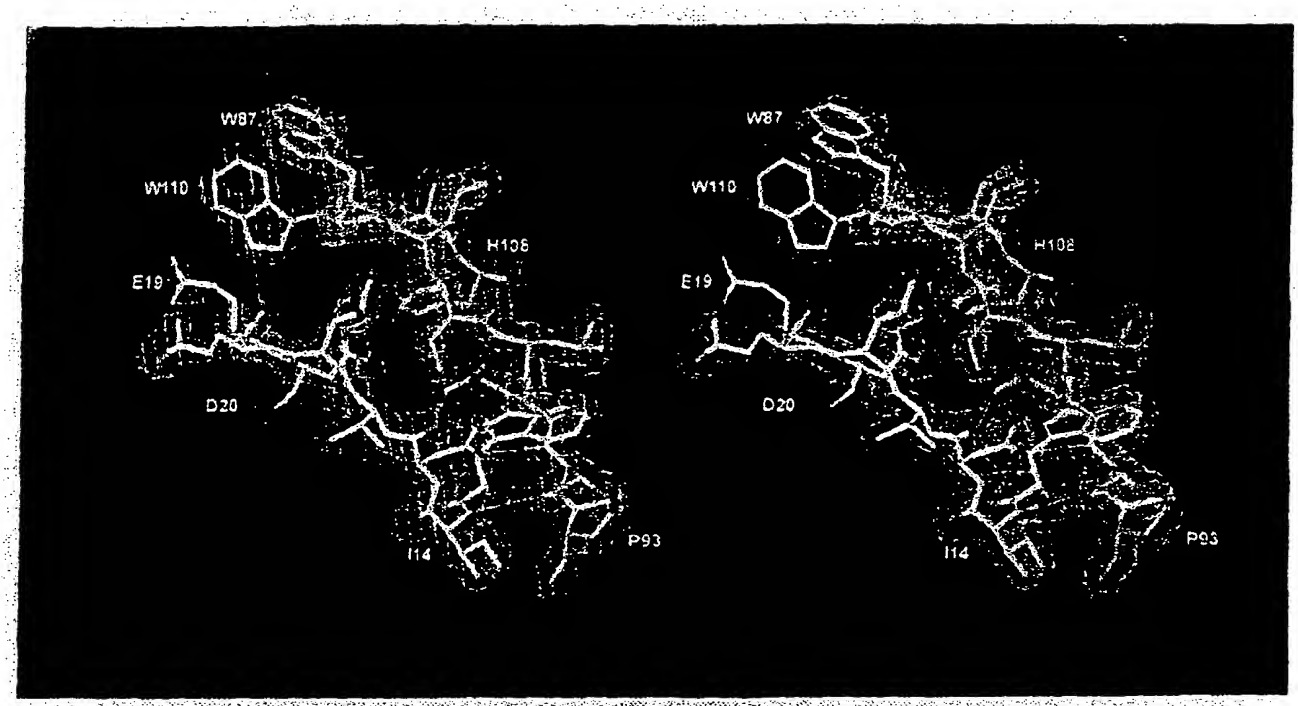

FIG. 4

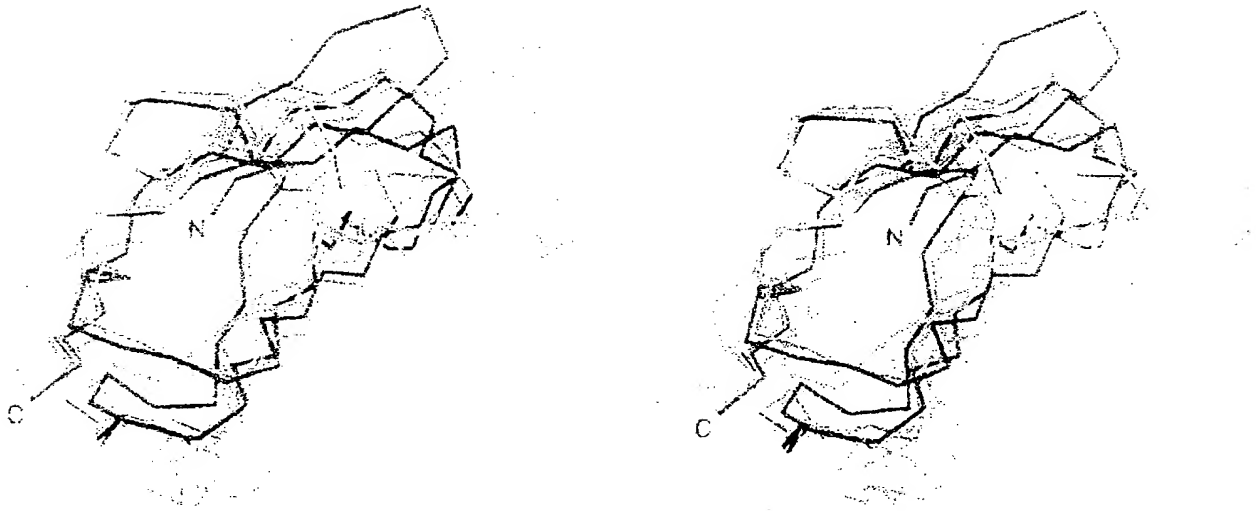

FIG. 5A

FIG. 5B

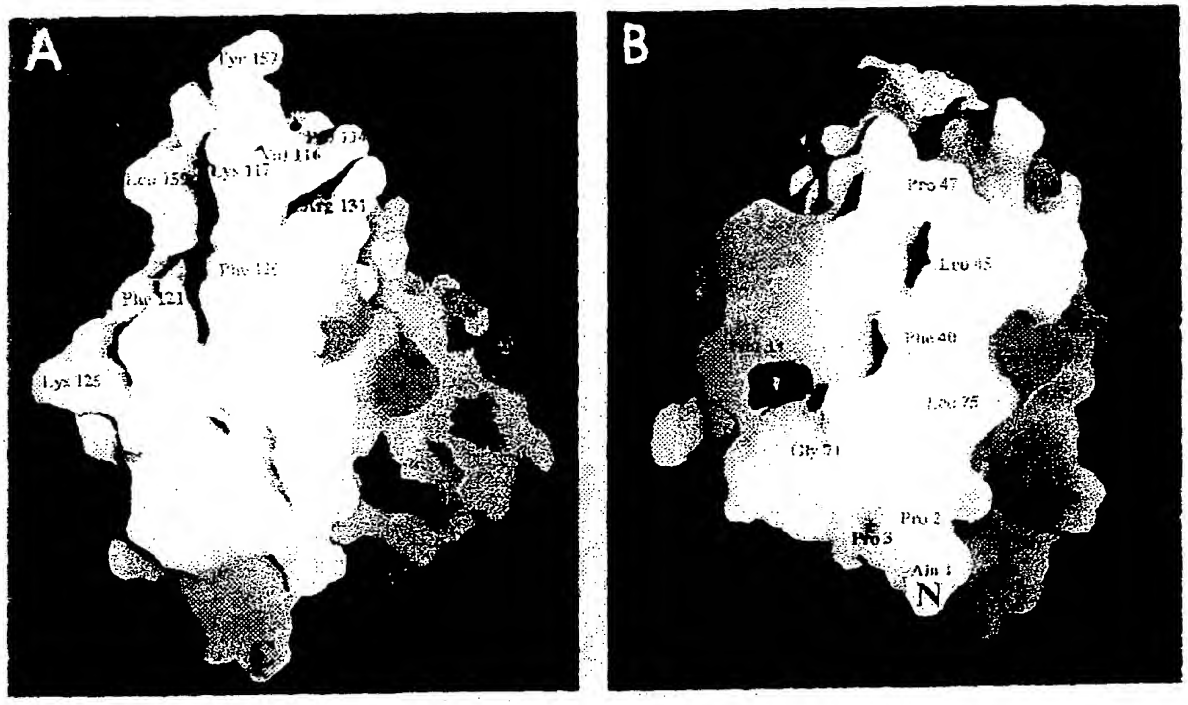
A
Tyr 152
Val 116
Lys 117
Ile 131
Phe 121
Lys 125
B
Pro 47
Leu 45
Phe 40
Leu 75
Gly 71
Pro 2
Pro 3
Asn 1
N

FIG. 6

FIG. 7

FIG. 8

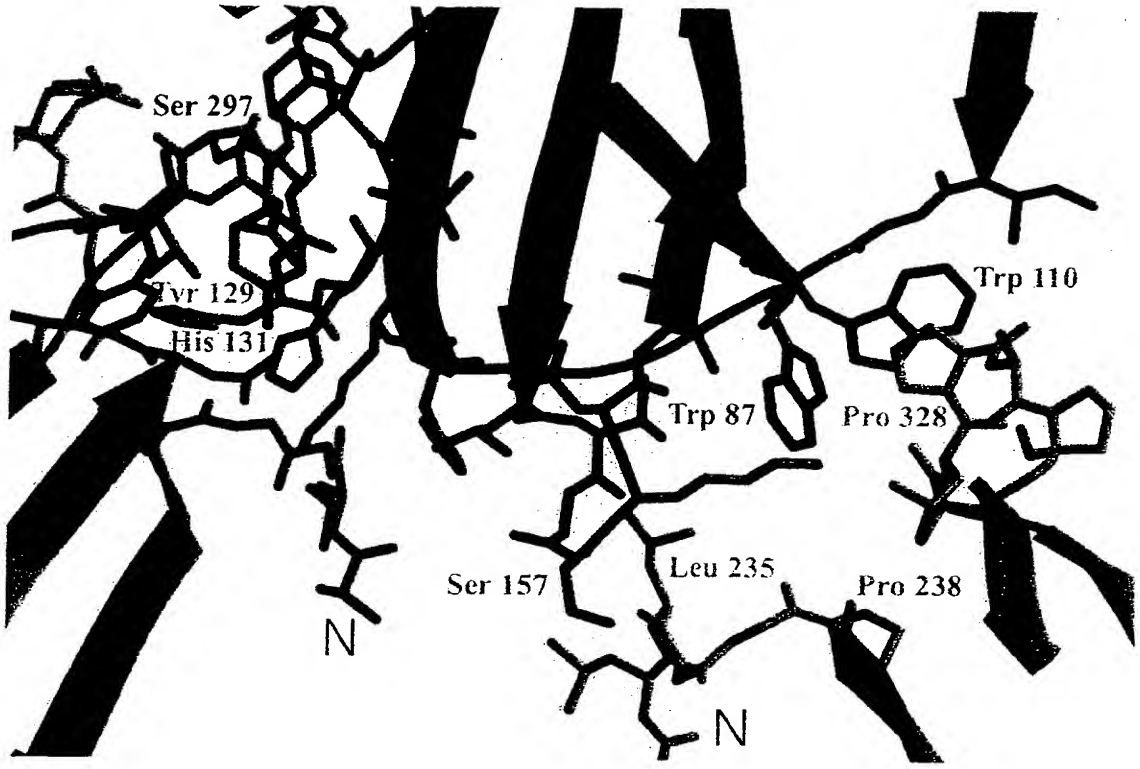
Ser 297
Tyr 129
His 131
Trp 110
Trp 87
Pro 328
Ser 157
Leu 235
Pro 238
N
N

FIG. 9

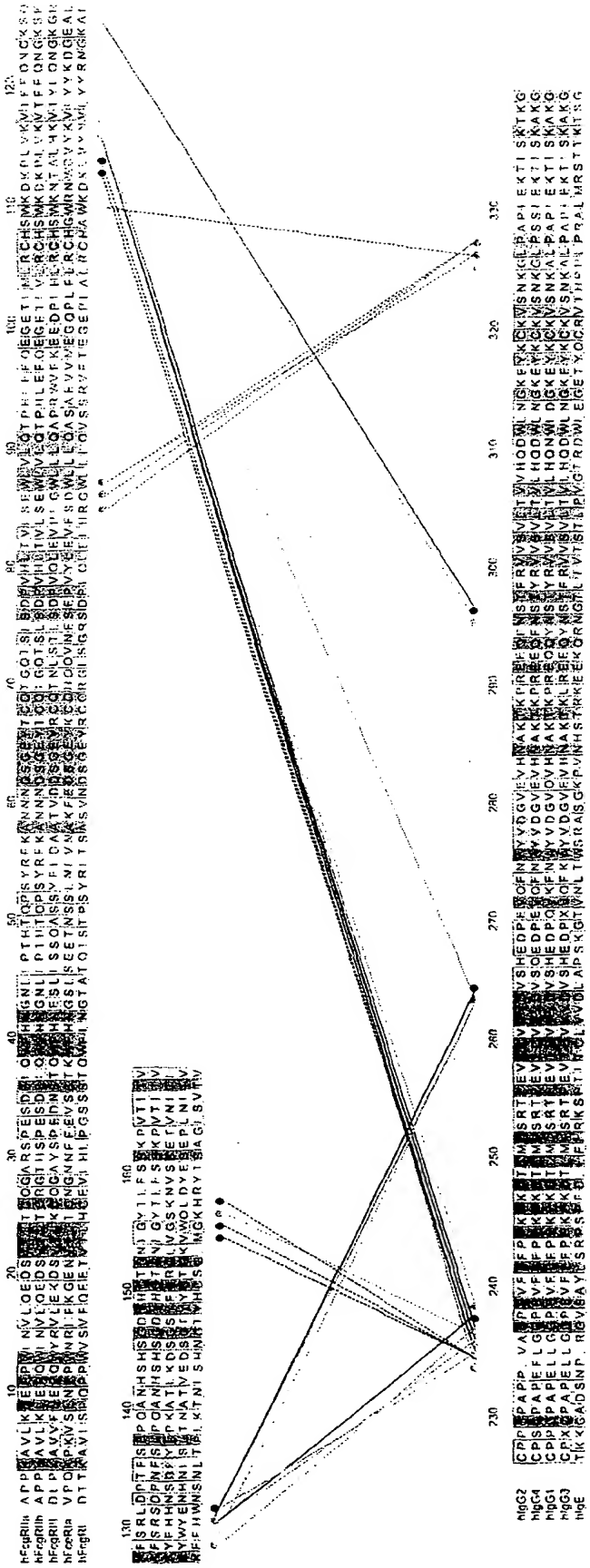

FIG. 10a

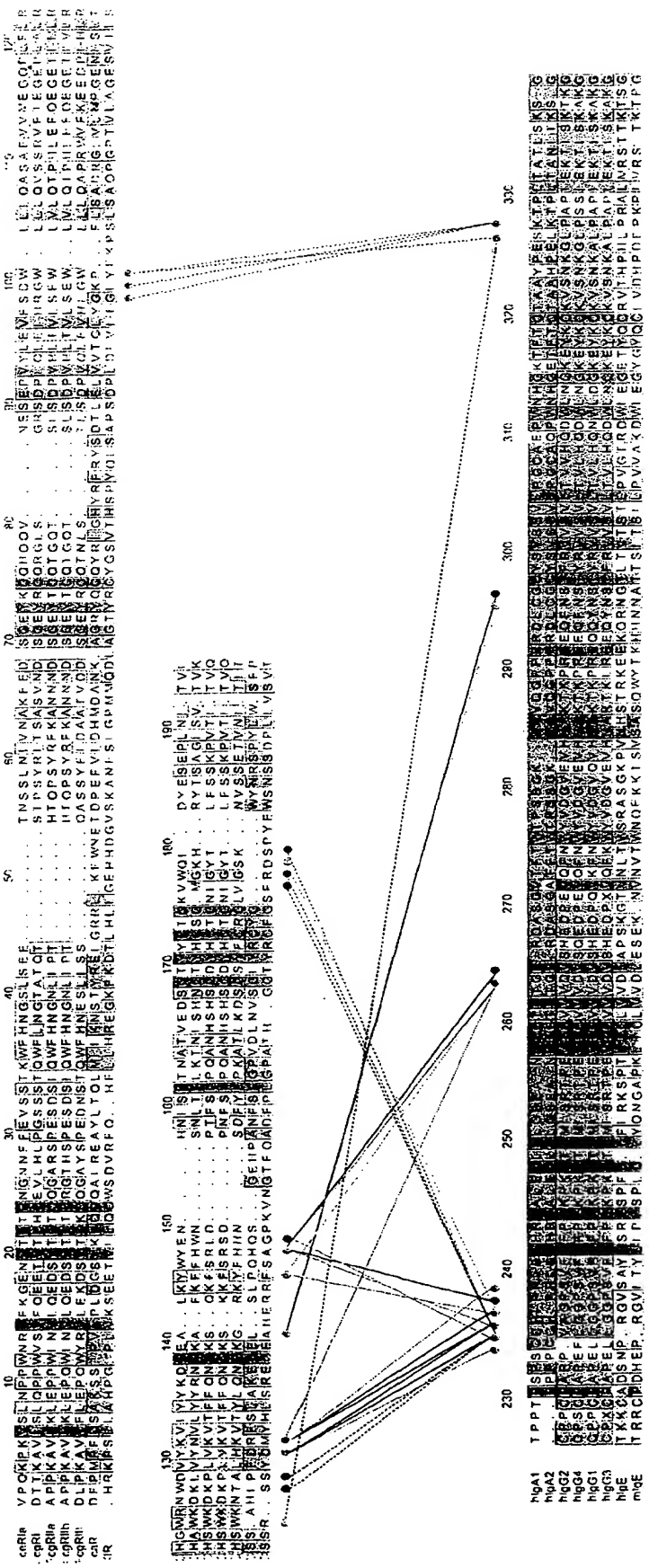

FIG. 10b

Alignment of the produced sFcγR, sFcεRIa and the short form of sFcεRII

```
sFcγRIIa        ---MAAPPKAVLKLEPP-WINVLQEDSVTLTCQGARSPESDSIQWFHN-GNLIPTHTQPS  55
sFcγRIIb        MGTPAAPPKAVLKLEPQ-WINVLQEDSVTLTCRGTHSPESDSIQWFHN-GNLIPTHTQPS  58
sFcγRIII        -MRTEDLPKAVVFLEPQ-WYSVLEKDSVTLKCQGAYSPEDNSTQWFHN-ESLISSQASSY  57
sFcγRI          --------MAVISLQPP-WVSVFQEETVTLHCEVLHLPGSSSTQWFLN-GTATQTSTPSY  50
sFcεRIa         ---MAVPQKPKVSLNPP-WNRIFKGENVTLTCNGNNFFEVSSTKWFHN-GSLSEETNSSL  55
sFcεRII         -MELQVSSGFVCNTCPEKWINFQRK------C---YYFGKGTKQWVHARYACDDMEGQLV  50
                                * * . .       *        .: :*.

sFcγRIIa        YRFKANNNDSG-EYTCQTGQTSLSDPVHLTVLSEWLV-LQTPHLEFQEGETIMLRCHSWK 113
sFcγRIIb        YRFKANNNDSG-EYTCQTGQTSLSDPVHLTVLSEWLV-LQTPHLEFQEGETIVLRCHSWK 116
sFcγRIII        FIDAATVNDSG-EYRCQTNLSTLSDPVQLEVHIGWLL-LQAPRWVFKEEDPIHLRCHSWK 115
sFcγRI          RITSASVNDSG-EYRCQRGLSGRSDPIQLEIHRGWLL-LQVSSRVFTEGEPLALRCHAWK 108
sFcεRIa         NIVNAKFEDSG-EYKCQHQQVNESEPVYLEVFSDWLL-LQASAEVVMEGQPLFLRCHGWR 113
sFcεRII         SIHSPEEQDFLTKHASHTGSWIGLRNLDLKGEFIWVDGSHVDYSNWAPGEPTS-RSQGED 109
                     .  :*   :: .:         : *     *:   :.        :.   *.:.

sFcγRIIa        DKPLVKVTFFQNGK-SQKFSRLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVQ 172
sFcγRIIb        DKPLVKVTFFQNGK-SKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLYSSKPVTITVQ 175
sFcγRIII        NTALHKVTYLQNGK-DRKYFHHNSDFHIPKATLKDSGSYFCRGLVGSKNVSSETVNITIT 174
sFcγRI          DKLVYNVLYYRNGK-AFKFFHWNSNLTILKTNISHNGTYHCSG-MGKHRYTSAGISVTVK 166
sFcεRIa         NWDVYKVIYYKDGE-ALKYWYENHNISITNATVEDSGTYYCTGKVWQLDYESEPLNITVI 172
sFcεRII         CVMMRGSGRWNDAFCDRKLGAWVCDRLATCTPPASEGSAESMGPDSRPDPDGRLPTPSAP 169
                   :      .:.    *            :    .*   . *        .   . :

sFcγRIIa        VP---------------------------------------------------------- 174
sFcγRIIb        APSSSPMGII-------------------------------------------------- 185
sFcγRIII        QG---------------------------------------------------------- 176
sFcγRI          ELFPAPVLNASVTSPLLEGNLVTLSCETKLLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQ 226
sFcεRIa         KAPREKYWLQF------------------------------------------------- 183
sFcεRII         LHS--------------------------------------------------------- 172

sFcγRIIa        ------------------------------------------
sFcγRIIb        ------------------------------------------
sFcγRIII        ------------------------------------------
sFcγRI          ILTARREDSGLYWCEAATEDGNVLKRSPELELQVLGLQLPTPV                  269
sFcεRIa         ------------------------------------------
sFcεRII          -----------------------------------------
```

FIG. 11

Alignment the produced sFcγR and sFcεRIa without sFcεRII

```
sFcγRIIa        ---MAAPPKAVLKLEPPWINVLQEDSVTLTCQGARSPESDSIQWFHNGNLIPTHTQPSYR 57
sFcγRIIb        MGTPAAPPKAVLKLEPQWINVLQEDSVTLTCRGTHSPESDSIQWFHNGNLIPTHTQPSYR 60
sFcγRIII        -MRTEDLPKAVVFLEPQWYSVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQASSYFI 59
sFcγRI          --------MAVISLQPPWVSVFQEETVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSYRI 52
sFcεRIa         ---MAVPQKPKVSLNPPWNRIFKGENVTLTCNGNNFFEVSSTKWFHNGSLSEETNSSLNI 57
                          . : *:* *  ::: :.*** *.       .* :** * .       .

sFcγRIIa        FKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIMLRCHSWKDKPL 117
sFcγRIIb        FKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIVLRCHSWKDKPL 120
sFcγRIII        DAATVNDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTAL 119
sFcγRI          TSASVNDSGEYRCQRGLSGRSDPIQLEIHRGWLLLQVSSRVFTEGEPLALRCHAWKDKLV 112
sFcεRIa         VNAKFEDSGEYKCQHQQVNESEPVYLEVFSDWLLLQASAEVVMEGQPLFLRCHGWRNWDV 117
                  *. :***** **       *:*: * :    **:**..    . * :.: ****.*::  :

sFcgRIIa        VKVTFFQNGKSQKFSRLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVQVP--- 174
sFcγRIIb        VKVTFFQNGKSKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLYSSKPVTITVQAPSSS 180
sFcγRIII        HKVTYLQNGKDRKYFHHNSDFHIPKATLKDSGSYFCRGLVGSKNVSSETVNITITQG--- 176
sFcγRI          YNVLYYRNGKAFKFFHWNSNLTILKTNISHNGTYHCSG-MGKHRYTSAGISVTVKELFPA 171
sFcεRIa         YKVIYYKDGEALKYWYENHNISITNATVEDSGTYYCTGKVWQLDYESEPLNITVIKAPRE 177
                 :* : ::*:  *:   :  : * ::. ...* *.* * :      *  :.:*:

sFcγRIIa        ------------------------------------------------------------
sFcγRIIb        PMGII-------------------------------------------------------- 185
sFcγRIII        ------------------------------------------------------------
sFcγRI          PVLNASVTSPLLEGNLVTLSCETKLLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTAR 231
sFcεRIa         KYWLQF------------------------------------------------------ 183

sFcγRIIa        ------------------------------------
sFcγRIIb        ------------------------------------
sFcγRIII        ------------------------------------
sFcγRI          REDSGLYWCEAATEDGNVLKRSPELELQVLGLQLPTPV 269
sFcεRIa         ------------------------------------
```

FIG. 12

Prot in sequences of the examples (mutated to yield soluble forms)

>sFcγRI SEQ ID NO: 1

MAVISLQPPWVSVFQEETVTLHCEVLHLPGSSSTQWFLNGTATQTSTPSYRITSASVNDS
GEYRCQRGLSGRSDPIQLEIHRGWLLLQVSSRVFTEGEPLALRCHAWKDKLVYNVLYYRN
GKAFKFFHWNSNLTILKTNISHNGTYHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTS
PLLEGNLVTLSCETKLLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWC
EAATEDGNVLKRSPELELQVLGLQLPTPV

SEQ ID NO: 2

>sFcγRIIa

MAAPPKAVLKLEPPWINVLQEDSVTLTCQGARSPESDSIQWFHNGNLIPTHTQPSYRFKA
NNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIMLRCHSWKDKPLVKV
TFFQNGKSQKFSRLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVQVP

SEQ ID NO: 3

>sFcγRIIb

MGTPAAPPKAVLKLEPQWINVLQEDSVTLTCRGTHSPESDSIQWFHNGNLIPTHTQPSYR
FKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIVLRCHSWKDKPL
VKVTFFQNGKSKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLYSSKPVTITVQAPSSS
PMGII

SEQ ID NO: 4

>sFcγRIII

MRTEDLPKAVVFLEPQWYSVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQASSYFID
AATVNDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALH
KVTYLQNGKDRKYFHHNSDFHIPKATLKDSGSYFCRGLVGSKNVSSETVNITITQG

SEQ ID NO: 5

>sFcεRIa

MAVPQKPKVSLNPPWNRIFKGENVTLTCNGNNFFEVSSTKWFHNGSLSEETNSSLNIVN
AKFEDSGEYKCQHQQVNESEPVYLEVFSDWLLLQASAEVVMEGQPLFLRCHGWRNWDVYK
VIYYKDGEALKYWYENHNISITNATVEDSGTYYCTGKVWQLDYESEPLNITVIKAPREKY
WLQF

SEQ ID NO: 6

>sFcεRII

MDTTQSLKQLEERAARNVSQVSKNLESHHGDQMTQKSQSTQISQELEELRAEQQRLKSQD
LELSWNLNGLQADLSSFKSQELNERNEASDLLERLREEVTKLRMELQVSSGFVCNTCPEK
WINFQRKCYYFGKGTKQWVHARYACDDMEGQLVSIHSPEEQDFLTKHASHTGSWIGLRNL
DLKGEFIWVDGSHVDYSNWAPGEPTSRSQGEDCVMMRGSGRWNDAFCDRKLGAWVCDRLA
TCTPPASEGSAESMGPDSRPDPDGRLPTPSAPLHS

DNA sequences of the examples (mutated to yi ld soluble forms)

>sFγRI SEQ ID NO: 7

```
   1 CATATGGCAG TGATCTCTTT GCAGCCTCCA TGGGTCAGCG TGTTCCAAGA GGAAACCGTA   60
  61 ACCTTGCACT GTGAGGTGCT CCATCTGCCT GGGAGCAGCT CTACACAGTG GTTTCTCAAT  120
 121 GGCACAGCCA CTCAGACCTC GACCCCCAGC TACAGAATCA CCTCTGCCAG TGTCAATGAC  180
 181 AGTGGTGAAT ACAGGTGCCA GAGAGGTCTC TCAGGGCGAA GTGACCCCAT ACAGCTGGAA  240
 241 ATCCACAGAG GCTGGCTACT ACTGCAGGTC TCCAGCAGAG TCTTCACGGA AGGAGAACCT  300
 301 CTGGCCTTGA GGTGTCATGC GTGGAAGGAT AAGCTGGTGT ACAATGTGCT TTACTATCGA  360
 361 AATGGCAAAG CCTTTAAGTT TTTCCACTGG AATTCTAACC TCACCATTCT GAAAACCAAC  420
 421 ATAAGTCACA ATGGCACCTA CCATTGCTCA GGCATGGGAA AGCATCGCTA CACATCAGCA  480
 481 GGAATATCTG TCACTGTGAA AGAGCTATTT CCAGCTCCAG TGCTGAATGC ATCTGTGACA  540
 541 TCCCCACTCC TGGAGGGGAA TCTGGTCACC CTGAGCTGTG AAACAAAGTT GCTCTTGCAG  600
 601 AGGCCTGGTT TGCAGCTTTA CTTCTCCTTC TACATGGGCA GCAAGACCCT GCGAGGCAGG  660
 661 AACACATCCT CTGAATACCA AATACTAACT GCTAGAAGAG AAGACTCTGG GTTATACTGG  720
 721 TGCGAGGCTG CCACAGAGGA TGGAAATGTC CTTAAGCGCA GCCCTGAGTT GGAGCTTCAA  780
 781 GTGCTTGGCC TCCAGTTACC AACTCCTGTC TAGTCTCGAG                        820
```

>sFcγRIIa SEQ ID NO: 8

```
   1 CATATGGCAG CTCCCCCAAA GGCTGTGCTG AAACTTGAGC CCCCGTGGAT CAACGTGCTC   60
  61 CAGGAGGACT CTGTGACTCT GACATGCCAG GGGGCTCGCA GCCCTGAGAG CGACTCCATT  120
 121 CAGTGGTTCC ACAATGGGAA TCTCATTCCC ACCCACACGC AGCCCAGCTA CAGGTTCAAG  180
 181 GCCAACAACA ATGACAGCGG GGAGTACACG TGCCAGACTG GCCAGACCAG CCTCAGCGAC  240
 241 CCTGTGCATC TGACTGTGCT TTCCGAATGG CTGGTGCTCC AGACCCCTCA CCTGGAGTTC  300
 301 CAGGAGGGAG AAACCATCAT GCTGAGGTGC CACAGCTGGA AGGACAAGCC TCTGGTCAAG  360
 361 GTCACATTCT TCCAGAATGG AAAATCCCAG AAATTCTCCC GTTTGGATCC CACCTTCTCC  420
 421 ATCCCACAAG CAAACCACAG TCACAGTGGT GATTACCACT GCACAGGAAA CATAGGCTAC  480
 481 ACGCTGTTCT CATCCAAGCC TGTGACCATC ACTGTCCAAG TGCCCTGAAG CTT         533
```

>sFcγRIIb SEQ ID NO: 9

```
   1 CCATGGGGAC ACCTGCAGCT CCCCCAAAGG CTGTGCTGAA ACTCGAGCCC CAGTGGATCA   60
  61 ACGTGCTCCA GGAGGACTCT GTGACTCTGA CATGCCGGGG GACTCACAGC CCTGAGAGCG  120
 121 ACTCCATTCA GTGGTTCCAC AATGGGAATC TCATTCCCAC CCACACGCAG CCCAGCTACA  180
 181 GGTTCAAGGC CAACAACAAT GACAGCGGGG AGTACACGTG CCAGACTGGC CAGACCAGCC  240
 241 TCAGCGACCC TGTGCATCTG ACTGTGCTTT CTGAGTGGCT GGTGCTCCAG ACCCCTCACC  300
 301 TGGAGTTCCA GGAGGGAGAA ACCATCGTGC TGAGGTGCCA CAGCTGGAAG GACAAGCCTC  360
 361 TGGTCAAGGT CACATTCTTC CAGAATGGAA AATCCAAGAA ATTTTCCCGT TCGGATCCCA  420
 421 ACTTCTCCAT CCCACAAGCA AACCACAGTC ACAGTGGTGA TTACCACTGC ACAGGAAACA  480
 481 TAGGCTACAC GCTGTACTCA TCCAAGCCTG TGACCATCAC TGTCCAAGCT CCCAGCTCTT  540
 541 CACCGATGGG GATCATTTAG GCTGTCGAC                                    569
```

>sFcγRIII SEQ ID NO: 10

```
   1 CATATGCGGA CTGAAGATCT CCCAAAGGCT GTGGTGTTCC TGGAGCCTCA ATGGTACAGC   60
  61 GTGCTTGAGA AGGACAGTGT GACTCTGAAG TGCCAGGGAG CCTACTCCCC TGAGGACAAT  120
 121 TCCACACAGT GGTTTCACAA TGAGAGCCTC ATCTCAAGCC AGGCCTCGAG CTACTTCATT  180
 181 GACGCTGCCA CAGTCAACGA CAGTGGAGAG TACAGGTGCC AGACAAACCT CTCCACCCTC  240
 241 AGTGACCCGG TGCAGCTAGA AGTCCATATC GGCTGGCTGT TGCTCCAGGC CCCTCGGTGG  300
 301 GTGTTCAAGG AGGAAGACCC TATTCACCTG AGGTGTCACA GCTGGAAGAA CACTGCTCTG  360
 361 CATAAGGTCA CATATTTACA GAATGGCAAA GACAGGAAGT ATTTTCATCA TAATTCTGAC  420
 421 TTCCACATTC CAAAAGCCAC ACTCAAAGAT AGCGGCTCCT ACTTCTGCAG GGGGCTTGTT  480
 481 GGGAGTAAAA ATGTGTCTTC AGAGACTGTG AACATCACCA TCACTCAAGG TTAAGCTT     538
```

>sFcεRIa SEQ ID NO: 11

```
   1 CATATGGCAG TCCCTCAGAA ACCTAAGGTC TCCTTGAACC CTCCATGGAA TAGAATATTT   60
  61 AAAGGAGAGA ATGTGACTCT TACATGTAAT GGGAACAATT TCTTTGAAGT CAGTTCCACC  120
 121 AAATGGTTCC ACAATGGCAG CCTTTCAGAA GAGACAAATT CAAGTTTGAA TATTGTGAAT  180
 181 GCCAAATTTG AAGACAGTGG AGAATACAAA TGTCAGCACC AACAAGTTAA TGAGAGTGAA  240
 241 CCTGTGTACC TGGAAGTCTT CAGTGACTGG CTGCTCCTTC AGGCCTCTGC TGAGGTGGTG  300
 301 ATGGAGGGCC AGCCCCTCTT CCTCAGGTGC CATGGTTGGA GGAACTGGGA TGTGTACAAG  360
 361 GTGATCTATT ATAAGGATGG TGAAGCTCTC AAGTACTGGT ATGAGAACCA CAACATCTCC  420
 421 ATTACAAATG CCACAGTTGA AGACAGTGGA ACCTACTACT GTACGGGCAA AGTGTGGCAG  480
 481 CTGGACTATG AGTCTGAGCC CCTCAACATT ACTGTAATAA AAGCTCCGCG TGAGAAGTAC  540
 541 TGGCTACAAT TTTAGGATCC                                             560
```

SEQ ID NO: 12

>sFcεRII

```
   1 CATATGGAGT TGCAGGTGTC CAGCGGCTTT GTGTGCAACA CGTGCCCTGA AAAGTGGATC    60
  61 AATTTCCAAC GGAAGTGCTA CTACTTCGGC AAGGGCACCA AGCAGTGGGT CCACGCCCGG   120
 121 TATGCCTGTG ACGACATGGA AGGGCAGCTG GTCAGCATCC ACAGCCCGGA GGAGCAGGAC   180
 181 TTCCTGACCA AGCATGCCAG CCACACCGGC TCCTGGATTG GCCTTCGGAA CTTGGACCTG   240
 241 AAGGGGGAGT TTATCTGGGT GGATGGGAGC CACGTGGACT ACAGCAACTG GGCTCCAGGG   300
 301 GAGCCCACCA GCCGGAGCCA GGGCGAGGAC TGCGTGATGA TGCGGGGCTC CGGTCGCTGG   360
 361 AACGACGCCT TCTGCGACCG TAAGCTGGGC GCCTGGGTGT GCGACCGGCT GGCCACATGC   420
 421 ACGCCGCCAG CCAGCGAAGG TTCCGCGGAG TCCATGGGAC CTGATTCAAG ACCAGACCCT   480
 481 GACGGCCGCC TGCCCACCCC CTCTGCCCCT CTCCACTCTT GAGCATGGAT CC           532
```

SEQ ID NO:13

human FcγRIIb2

```
1     ggctgtgact gctgtgctct gggcgccact cgctccaggg agtgatggga atcctgtcat
61    ttttacctgt ccttgccact gagagtgact gggctgactg caagtccccc cagccttggg
121   gtcatatgct tctgtggaca gctgtgctat tcctggctcc tgttgctggg acacctgcag
181   ctcccccaaa ggctgtgctg aaactcgagc cccagtggat caacgtgctc caggaggact
241   ctgtgactct gacatgccgg gggactcaca gccctgagag cgactccatt cagtggttcc
301   acaatgggaa tctcattccc acccacacgc agcccagcta caggttcaag gccaacaaca
361   atgacagcgg ggagtacacg tgccagactg gccagaccag cctcagcgac cctgtgcatc
421   tgacagtgct ttctgagtgg ctggtgctcc agacccctca cctggagttc caggagggag
481   aaaccatcgt gctgaggtgc cacagctgga aggacaagcc tctggtcaag gtcacattct
541   tccagaatgg aaaatccaag aaattttccc gttcggatcc caacttctcc atcccacaag
601   caaaccacag tcacagtggt gattaccatt gcacaggaaa cataggctac acgctgtact
661   catccaagcc tgtgaccatc actgtccaag ctcccagctc ttcaccgatg gggatcattg
721   tggctgtggt cactgggatt gctgtagctg ccattgttgc tgctgtagtg gccttgatct
781   actgcaggaa aaagcggatt tcagccaatc ccactaatcc tgatgaggct gacaaagttg
841   gggctgagaa cacaatcacc tattcacttc tcatgcaccc ggatgctctg gaagagcctg
901   atgaccagaa ccgtatttag tctccattgt cttgcattgg gatttgagaa gaaatcagag
961   agggaagatc tggtatttcc tggcctaaat tccccttggg gaggacaggg agatgctgca
1021  gttccaaaag agaaggtttc ttccagagtc atctacctga gtcctgaagc tccctgtcct
1081  gaaagccaca gacaatatgg tcccaaatgc ccgactgcac cttctgtgct tcagctcttc
1141  ttgacatcaa ggctcttccg ttccacatcc acacagccaa tccaattaat caaaccactg
1201  ttattaacag ataatagcaa cttgggaaat gcttatgtta caggttacgt gagaacaatc
1261  atgtaaatct atatgatttc agaaatgtta aaatagacta acctctacca gcacattaaa
1321  agtgattgtt tctgggtgat aaaattattg atgattttta ttttctttat ttttctataa
1381  agatcatata ttacttttat aataaaacat tataaaaac
```

SEQ ID NO:14

human FcεRIa

```
1     agatctcagc acagtaagca ccaggagtcc atgaagaaga tggctcctgc catggaatcc
61    cctactctac tgtgtgtagc cttactgttc ttcgctccag atggcgtgtt agcagtccct
121   cagaaaccta aggtctcctt gaaccctcca tggaatagaa tatttaaagg agagaatgtg
181   actcttacat gtaatgggaa caatttcttt gaagtcagtt ccaccaaatg gttccacaat
241   ggcagccttt cagaagagac aaattcaagt ttgaatattg tgaatgccaa atttgaagac
301   agtggagaat acaaatgtca gcaccaacaa gttaatgaga gtgaacctgt gtacctggaa
361   gtcttcagtg actggctgct ccttcaggcc tctgctgagg tggtgatgga gggccagccc
421   ctcttcctca ggtgccatgg ttggaggaac tgggatgtgt acaaggtgat ctattataag
481   gatggtgaag ctctcaagta ctggtatgag aaccacaaca tctccattac aaatgccaca
541   gttgaagaca gtggaaccta ctactgtacg ggcaaagtgt ggcagctgga ctatgagtct
601   gagcccctca acattactgt aataaaagct ccgcgtgaga agtactggct acaatttttt
661   atcccattgt tggtggtgat tctgtttgct gtggacacag gattatttat ctcaactcag
721   cagcaggtca catttctctt gaagattaag agaaccagga aaggcttcag acttctgaac
781   ccacatccta agccaaaccc caaaaacaac tgatataatt aactcaagaa atatttgcaa
841   cattagtttt tttccagcat cagcaattgc tactcaattg tcaaacacag cttgcaatat
901   acatagaaac gtctgtgctc aaggatttat agaaatgctt cattaaactg agtgaaactg
961   attaagtggc atgtaatagt aagtgctcaa ttaacattgg ttgaataaat gagagaatga
1021  atagattcat ttattagcat ttgtaaaaga gatgttcaat ttagatct
```

SEQ ID NO:15

human mRNA for high affinity Fc receptor (FcγRI)

```
1     gacagatttc actgctccca ccagcttgga gacaacatgt ggttcttgac aactctgctc
61    ctttgggttc cagttgatgg gcaagtggac accacaaagg cagtgatctc tttgcagcct
121   ccatgggtca gcgtgttcca agaggaaacc gtaaccttgc actgtgaggt gctccatctg
181   cctgggagca gctctacaca gtggtttctc aatggcacag ccactcagac ctcgaccccc
241   agctacagaa tcacctctgc cagtgtcaat gacagtggtg aatacaggtg ccagagaggt
301   ctctcagggc gaagtgaccc catacagctg gaaatccaca gaggctggct actactgcag
361   gtctccagca gagtcttcac ggaaggagaa cctctggcct tgaggtgtca tgcgtggaag
421   gataagctgg tgtacaatgt gctttactat cgaaatggca aagcctttaa gtttttccac
481   tggaattcta acctcaccat tctgaaaacc aacataagtc acaatggcac ctaccattgc
541   tcaggcatgg gaaagcatcg ctacacatca gcaggaatat ctgtcactgt gaaagagcta
601   tttccagctc cagtgctgaa tgcatctgtg acatccccac tcctggaggg gaatctggtc
661   accctgagct gtgaaacaaa gttgctcttg cagaggcctg gtttgcagct ttacttctcc
721   ttctacatgg gcagcaagac cctgcgaggc aggaacacat cctctgaata ccaaatacta
781   actgctagaa gagaagactc tgggttatac tggtgcgagg ctgccacaga ggatggaaat
841   gtccttaagc gcagccctga gttggagctt caagtgcttg gcctccagtt accaactcct
901   gtctggtttc atgtcctttt ctatctggca gtgggaataa tgtttttagt gaacactgtt
961   ctctgggtga caatacgtaa agaactgaaa agaaagaaaa agtgggattt agaaatctct
1021  ttggattctg gtcatgagaa gaaggtaact tccagccttc aagaagacag acatttagaa
1081  gaagagctga aatgtcagga acaaaaagaa gaacagctgc aggaaggggt gcaccggaag
1141  gagccccagg gggccacgta gcagcggctc agtgggtggc catcgatctg gaccgtcccc
1201  tgcccacttg ctccccgtga gcactgcgta caaacatcca aaagttcaac aacaccagaa
1261  ctgtgtgtct catggtatgt aactcttaaa gcaaataaat gaactgactt caaaaaaaaa
1321  a
```

SEQ ID NO:16

human FcγRIIa

```
1     cccaaatgtc tcagaatgta tgtcccagaa acctgtggct gcttcaacca ttgacagttt
61    tgctgctgct ggcttctgca gacagtcaag ctgcagctcc cccaaaggct gtgctgaaac
121   ttgagccccc gtggatcaac gtgctccagg aggactctgt gactctgaca tgccaggggg
181   ctcgcagccc tgagagcgac tccattcagt ggttccacaa tgggaatctc attcccaccc
241   acacgcagcc cagctacagg ttcaaggcca acaacaatga cagcggggag tacacgtgcc
301   agactggcca gaccagcctc agcgaccctg tgcatctgac tgtgctttcc gaatggctgg
361   tgctccagac ccctcacctg gagttccagg agggagaaac catcatgctg aggtgccaca
421   gctggaagga caagcctctg gtcaaggtca cattcttcca gaatggaaaa tcccagaaat
481   tctcccgttt ggatcccacc ttctccatcc cacaagcaaa ccacagtcac agtggtgatt
541   accactgcac aggaaacata ggctacacgc tgttctcatc caagcctgtg accatcactg
601   tccaagtgcc cagcatgggc agctcttcac caatggggat cattgtggct gtggtcattg
661   cgactgctgt agcagccatt gttgctgctg tagtggcctt gatctactgc aggaaaaagc
721   ggatttcagc caattccact gatcctgtga aggctgccca atttgagcca cctggacgtc
781   aaatgattgc catcagaaag agacaacttg aagaaaccaa caatgactat gaaacagctg
841   acggcggcta catgactctg aaccccaggg cacctactga cgatgataaa aacatctacc
901   tgactcttcc tcccaacgac catgtcaaca gtaataacta aagagtaacg ttatgccatg
961   tggtcatact ctcagcttgc tgatggatga caaaaagagg ggaattgtta aaggaaaatt
1021  taaatggaga ctggaaaaat cctgagcaaa caaaaccacc tggcccttag aaatagcttt
1081  aactttgctt aaactacaaa cacaagcaaa acttcacggg gtcatactac atacaagcat
1141  aagcaaaact taacttggat catttctggt aaatgcttat gttagaaata agacaacccc
1201  agccaatcac aagcagccta ctaacatata attaggtgac tagggacttt ctaagaagat
1261  acctaccccc aaaaaacaat tatgtaattg aaaaccaacc gattgccttt attttgcttc
1321  cacatttttcc caataaatac ttgcctgtga cattttgcca ctggaacact aaacttcatg
1381  aattgcgcct cagatttttc ctttaacatc ttttttttt ttgacagagt ctcaatctgt
1441  tacccaggct ggagtgcagt ggtgctatct tggctcactg caaacccgcc tcccaggttt
1501  aagcgattct tatgcctcag cctcccagta gctgggatta gaggcatgtg ccatcatacc
1561  cagctaattt ttgtattttt tatttttttat ttttagtaga gacagggttt cgcaatgttg
1621  gccaggccga tctcgaactt ctggcctcta gcgatctgcc cgcctcggcc tcccaaagtg
1681  ctgggatgac cgcatcagcc ccaatgtcca gcctctttaa catcttcttt cctatgccct
1741  ctctgtggat ccctactgct ggtttctgcc ttctccatgc tgagaacaaa atcacctatt
1801  cactgcttat gcagtcggaa gctccagaag aacaaagagc ccaattacca gaaccacatt
1861  aagtctccat tgttttgcct tgggatttga gaagagaatt agagaggtga ggatctggta
1921  tttcctggac taaattccct tggggaagac gaagggatgc tgcagttcca aaagagaagg
1981  actcttccag agtcatctac ctgagtccca aagctccctg tcctgaaagc cacagacaat
2041  atggtcccaa atgactgact gcaccttctg tgcctcagcc gttcttgaca tcaagaatct
2101  tctgttccac atccacacag ccaatacaat tagtcaaacc actgttatta acagatgtag
2161  caacatgaga aacgcttatg ttacaggtta catgagagca atcatgtaag tctatatgac
2221  ttcagaaatg ttaaaataga ctaacctcta acaacaaatt aaaagtgatt gtttcaaggt
2281  gatgcaatta ttgatgacct attttatttt tctataatga tcatatatta cctttgtaat
2341  aaaacattat aaccaaaac
```

SEQ ID NO:17

human FcγRIII

```
1    tctttggtga cttgtccact ccagtgtggc atcatgtggc agctgctcct cccaactgct
61   ctgctacttc tagtttcagc tggcatgcgg actgaagatc tcccaaaggc tgtggtgttc
121  ctggagcctc aatggtacag cgtgcttgag aaggacagtg tgactctgaa gtgccaggga
181  gcctactccc ctgaggacaa ttccacacag tggtttcaca atgagagcct catctcaagc
241  caggcctcga gctacttcat tgacgctgcc acagtcaacg acagtggaga gtacaggtgc
301  cagacaaacc tctccaccct cagtgacccg gtgcagctag aagtccatat cggctggctg
361  ttgctccagg cccctcggtg ggtgttcaag gaggaagacc ctattcacct gaggtgtcac
421  agctggaaga acactgctct gcataaggtc acatatttac agaatggcaa agacaggaag
481  tattttcatc ataattctga cttccacatt ccaaaagcca cactcaaaga tagcggctcc
541  tacttctgca gggggcttgt tgggagtaaa aatgtgtctt cagagactgt gaacatcacc
601  atcactcaag gtttggcagt gtcaaccatc tcatcattct ctccacctgg gtaccaagtc
661  tctttctgct tggtgatggt actccttttt gcagtggaca caggactata tttctctgtg
721  aagacaaaca tttgaagctc aacaagagac tggaaggacc ataaacttaa atggagaaag
781  gaccctcaag acaaatgacc cccatcccat gggagtaata agagcagtgg cagcagcatc
841  tctgaacatt tctctggatt tgcaacccca tcatcctcag gcctctc
```

SEQ ID NO: 18

human FcεRII

```
        1 ctcctgctta aacctctgtc tctgacggtc cctgccaatc gctctggtcg
accccaacac
       61 actaggagga cagacacagg ctccaaactc cactaagtga ccagagctgt gattgtgccc
      121 gctgagtgga ctgcgttgtc agggagtgag tgctccatca tcgggagaat ccaagcagga
      181 ccgccatgga ggaaggtcaa tattcagaga tcgaggagct tcccaggagg cggtgttgca
      241 ggcgtgggac tcagatcgtg ctgctggggc tggtgaccgc cgctctgtgg gctgggctgc
      301 tgactctgct tctcctgtgg cactgggaca ccacacagag tctaaaacag ctggaagaga
      361 gggctgcccg gaacgtctct caagtttcca agaacttgga aagccaccac ggtgaccaga
      421 tggcgcagaa atcccagtcc acgcagattt cacaggaact ggaggaactt cgagctgaac
      481 agcagagatt gaaatctcag gacttggagc tgtcctggaa cctgaacggg cttcaagcag
      541 atctgagcag cttcaagtcc caggaattga acgagaggaa cgaagcttca gatttgctgg
      601 aaagactccg ggaggaggtg acaaagctaa ggatggagtt gcaggtgtcc agcggctttg
      661 tgtgcaacac gtgccctgaa aagtggatca atttccaacg gaagtgctac tacttcggca
      721 agggcaccaa gcagtgggtc cacgcccggt atgcctgtga cgacatggaa gggcagctgg
      781 tcagcatcca cagcccggag gagcaggact tcctgaccaa gcatgccagc cacaccggct
      841 cctggattgg ccttcggaac ttggacctga agggagagtt tatctgggtg gatgggagcc
      901 atgtggacta cagcaactgg gctccagggg agcccaccag ccggagccag ggcgaggact
      961 gcgtgatgat gcggggctcc ggtcgctgga acgacgcctt ctgcgaccgt aagctgggcg
     1021 cctgggtgtg cgaccggctg gccacatgca cgccgccagc cagcgaaggt tccgcggagt
     1081 ccatgggacc tgattcaaga ccagaccctg acggccgcct gcccaccccc tctgcccctc
     1141 tccactcttg agcatggata cagccaggcc cagagcaaga ccctgaagac ccccaaccac
     1201 ggcctaaaag cctctttgtg gctgaaaggt ccctgtgaca ttttctgcca cccaaacgga
     1261 ggcagctgac acatctcccg ctcctctatg gcccctgcct tcccaggagt acaccccaac
     1321 agcaccctct ccagatggga gtgcccccaa cagcaccctc tccagatgag agtacacccc
     1381 aacagcaccc tctccagatg cagccccatc tcctcagcac cccaggacct gagtatcccc
     1441 agctcaggtg gtgagtcctc ctgtccagcc tgcatcaata aaatggggca gtgatggcct
     1501 ccc
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INTERNATIONAL SEARCH REPORT

..ternational application No.
PCT/EP 99/09440

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 31-38
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Ir. ational Application No
PCT/EP 99/09440

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C12N1/21 C12N15/70 G01N33/53
G01N33/68 A61K38/17 C07K17/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	FR 2 739 560 A (ROUSSEL UCLAF) 11 April 1997 (1997-04-11) page 3, last line -page 6, line 28	1-23 24,26, 28-30
X Y	EP 0 614 978 A (ROUSSEL-UCLAF) 14 September 1994 (1994-09-14) page 3, line 1 -page 7, line 33; examples	1,2, 4-13,16, 21-23 24,26, 28-30

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 2000	12/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Ir. ational Application No
PCT/EP 99/09440

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 623 053 A (LOUIS N. GASTINEL ET AL.) 22 April 1997 (1997-04-22)	1,4,6, 8-13, 39-42
Y	column 5, line 60 -column 8, line 67; examples	5,7,24, 26,28-30
X	WO 96 40199 A (UNIVERSITY OF PENNSYLVANIA) 19 December 1996 (1996-12-19)	1,2,4,6, 8-16, 19-22
Y	page 22, line 24 -page 24, line 14; claims 13-16,36-39; examples I,II	5,7,24, 26,28-30
X	WO 95 09002 A (UNIVERSITY OF PENNSYLVANIA) 6 April 1995 (1995-04-06)	1,2,4,6, 8-13,16, 21,39,40
Y	page 15, line 4 -page 16, line 22; claims 28-31; examples I,II	5,7,24, 26,28-30
X	EP 0 791 653 A (SCHERING BIOTECH CORPORATION) 27 August 1997 (1997-08-27)	1,2,4,6, 8-11,21, 22
Y	column 5, line 56 -column 10, line 1; example III	5,7,24, 26,28-30
X	JÉRÔME GALON ET AL.: "Ligands and biological activities of soluble Fcgamma receptors" IMMUNOLOGY LETTERS, vol. 44, January 1995 (1995-01), pages 175-181, XP000574018	1-4,6, 8-13,15, 16,19-22
Y	page 175, right-hand column, last paragraph -page 177, left-hand column, paragraph 1 page 179, right-hand column, paragraph 3 -page 180, right-hand column, paragraph 3	5,7,24, 26,28-30
X	EP 0 321 842 A (KISHIMOTO, TADAMITSU) 28 June 1989 (1989-06-28)	1,2, 4-19,21, 22
Y	column 2, line 10 -column 9, line 43; figure 4; examples B,,1-3,6,,7	24,26, 28,30

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/09440

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y Y	EP 0 259 615 A (KISHIMOTO, TADAMITSU) 16 March 1988 (1988-03-16) page 2, line 1 -page 37, line 13 JANET M. ALLEN ET AL.: "Isolation and expression of functional high-affinity Fc receptor complementary DNAs" JOURNAL OF CRYSTAL GROWTH, vol. 93, no. 1-4, November 1988 (1988-11) - December 1988 (1988-12), pages 378-381, XP000002750 Amsterdam, NL abstract; figure 4 WILHELM P. BURMEISTER ET AL.: "Crystal structure at 2.2A resolution of the MHC-related neonatal Fc receptor" NATURE, vol. 372, no. 6504, 24 November 1994 (1994-11-24), pages 336-343, XP002135452 LONDON GB the whole document	1,2, 4-15, 17-19, 21,22 24,26, 28-30 5,7 24,26, 28-30
P,X	WO 99 05271 A (GOULD, HANNAH ET AL.) 4 February 1999 (1999-02-04) page 4, line 6 - line 19 page 5, line 22 -page 6, line 2 page 7, line 22 -page 8, line 23 page 10, line 31 -page 11, line 31	1,2,4,6, 8-15,21, 22,39

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 31-38

Present claims 31-38 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is not to be found, however, for any specific example of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, no search has been carried out for claims 31-38.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/EP 99/09440

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INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/EP 99/09440

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